NUTRITIONAL DEFICIENCIES

DIAGNOSIS AND TREATMENT

BY

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16 Illustrations

SECOND EDITION



Philadelphia Montreal London

J B LIPPINCOTT COMPANY

SECOND EDITION

PRINTED IN THE UNITED STATES OF AMERICA

TO MY WIFE

LOLA DEA YOUMANS

Preface to the Second Edition

THE preparation of a second edition of this book has provided a welcome opportunity to correct some errors found in the first It has also been possible carefully to examine every part and to determine the need for alterations or the addition of new material In the 18 months since the book first appeared rapid advance in the field of nutrition has continued, but that which has been applicable clinically has not been great However, additional material has been added on the changes in the eye in vitamin A deficiency and the use of blood vitamin A determinations The sections on the diagnosis of thiamin, nicotinic acid, and vitamin C deficiency have been largely rewritten and new material added Current ideas on the vascularization of the cornea in relation to riboflavin deficiency are indicated Chapter 13, on other possible nutritional deficiencies, has been almost completely rewritten with respect to the present status of other vitamins which may be significant in human nutrition

To the appendix has been added a table of the recommended allowances of the Food and Nutrition Board of the National Research Council, and in general throughout the book the suggested allowances of the various nutritive factors have been altered when necessary to correspond to those of the Board It is realized that these are probably generous, but for application in clinical medicine a liberal factor of safety is desirable In the appendix the section on Diagnostic Methods has had considerable revision and some new material has been added, mainly the method for deter

mining pyruvic acid in the blood The determination of thiamin by the method of Melnick and Field has been omitted Though satisfactory in expert hands it is not well adapted to clinical laboratory work

The authors are grateful for the favorable attention the book has received and for the many kind and complimentary notices They are equally grateful for the constructive criticisms and helpful suggestions which have been given Many are incorporated in the changes we have made Wherever sound criticisms have not been followed equally sound reasons for failure to do so have existed

We wish to express our thanks to our many associates and others who have given helpful advice, to Mrs Macon Somerville for her secretarial aid, and to the National Research Council for permission to publish the Table of Recommended Allowances We are likewise grateful to the J B Lippincott Company for their patient and courteous assistance

JOHN B YOUMANS

Preface to the First Edition

Rapid progress has greatly increased our understanding of the clinical manifestations of nutritional deficiencies, their diagnosis and treatment. But, this rapid progress has scattered the information in widely separated places, often difficult of access, bewilderingly mixed with experimental laboratory data and in a state of confusion which makes the acquisition, interpretation, and use of this information difficult for the physician. This book has been written to bring together in a useful and critical fashion such of this information as is necessary and helpful for a proper understanding and management of nutritional deficiencies in practice.

Although the vitamins occupy a large part of the book, other essential food factors, whose deficiency leads to recognizable signs and symptoms, are included to complete a logical grouping of diseases whose main characteristic is that they are caused by the *absence*, rather than the *presence*, of the etiologic agent. Deficiencies of calories (starvation) and water (dehydration) have, however, not been included because they are not ordinarily considered with deficiency diseases. A discussion of the nature, function, and sources of the various food factors is given to provide the necessary background for an understanding of the diseases, which a deficiency of them causes, but the book is not a treatise on nutrition. Neither is it a book on dietetics although the proper use of foods as sources of the essential dietary factors in the prevention and treatment of deficiency diseases is discussed. With few exceptions no material which is based solely on observations on animals has been included for

reasons which are discussed in Chapter 1 The few instances where it has been done have been clearly indicated

In so far as possible the various clinical phases of these nutritional diseases, etiology, pathogenesis, diagnosis, et cetera, have been presented in a uniform manner and in a style made familiar by the usual text books of medicine In the discussion of treatment, however, prevention has been given the added emphasis it deserves by placing it before curative treatment The details of the technic of the various laboratory tests are given in the appendix To have included them in the body of the book would have lessened the force of the general discussion To have excluded them would have lessened greatly the intended value of the book The appendix includes also tables of units, tables for the con version of units, and certain limited lists of foods

Relatively few bibliographic references have been given in spite of an enormous and growing literature on nutri tion Recent developments have made much of this enor mous literature mainly of historical interest Much of it deals with experimental studies which have no direct ap plication to clinical medicine A great deal of it is scattered in isolated fragments which must be interpreted in the light of many other fragments and to include all these references would require a bibliography unsuited to a book of this kind

The speed of advance in this field at this time makes it inevitable that changes and new developments will occur between the time a book on nutritional disease is written and the time it appears in print To avoid this, the inclina tion is to include material which is so recent that it may not have received sufficient confirmation On the other hand too great delay in recording new discoveries makes the book of little use With these facts in mind I have tried to avoid either extreme and to present material which is not so new as to be too controversial yet give as much helpful and re

liable new information as possible In almost all cases such new material has had the test of personal experience in my own practice Some risk of prediction must be taken and some dependence must be placed on the critical judgment of practitioners whose experience will, in the long run, de termine the worth of much that is proposed

To acknowledge individually the help of all who have contributed to the writing of this book would require the listing of nearly all who have contributed to the literature of nutrition in the last two decades and many from earlier times My special thanks are due to many of my colleagues who have contributed generously of time and effort in giv ing information, advice and criticisms In particular, I am grateful to my associate, Dr E White Patton, who has pre pared the material for the appendix and in my absence has read the proof and performed many other arduous tasks in connection with the details of publication And, through it all, I have had the untiring, willing help of my secretary, Mrs Elma Lee West, without which such work would be impossible and to whom I am deeply grateful

J B Y

Chapter

amounts and if the requirement for iron seems large at 10 mg the vitamin C needs are 5 to 10 times as great

A third characteristic is that they are fundamentally related to the nutrition of many, if not all, of the cells of the body They play an essential and basic rôle in life processes This characteristic appears to, but does not, contradict a fourth characteristic which is their specificity in relation to disease The fact that deficiencies of individual factors cause specific and distinctive disease patterns does not mean that only certain tissues are affected (Many accessory food factors have a non specific growth effect) This specific effect can be attributed to the relatively greater effect of such a deficiency on the more vulnerable tissues or organs

A fifth characteristic is that they cannot be formed or synthesized by the body This, which is perhaps the most distinguishing feature, is not an absolute characteristic, however, and there is a considerable variation in different species in this respect Thus, the rat, and other animals can synthesize vitamin C Man and the guinea pig cannot Man can make vitamin A from carotene, the final step in synthesis, but cannot, nor, as far as is known, can any other animal, synthesize carotene Man can obtain vitamin K from his intestinal tract where it is formed by the action of bacteria, but this can hardly be called synthesis He can form vitamin D in the skin provided he is exposed to ultraviolet radiation Aside from these very limited abilities however, he is dependent for his vitamins, as he is for minerals and certain amino acids on the supply in his food or drink Szent Gyorgyi[1] speculates that the loss of ability to synthesize vitamins has come about in an evolutionary fashion because an abundant exogenous supply has made synthesis unnecessary, a sort of ' disuse atrophy ' of function Although man and other primates are possibly more dependent on an exogenous supply than are other animals, there are few studies in comparative physiology dealing with this topic

A sixth characteristic of the essential accessory food factors is their lack of toxicity or potentiality for harm This is, of course, consistent with their place as fundamental factors in the life of living cells As such, an inadequate supply of them is inimical to the cell, an optimal supply favors optimal growth and function, while an excess is without effect and is eliminated without injury This means that in general such substances are without pharmacologic action However, this characteristic also is relative and not absolute, and is variable with respect to the different food factors Enormous amounts of some vitamins may be taken without any ill effect except for certain non specific reactions, such as that caused by the acidity of ascorbic acid (vitamin C) Ascorbic acid can cause untoward effects by its *acidity* just as can other acids However, some vitamins exhibit a toxic action in doses which, while very large, are yet near the upper range of doses used in prevention or treatment of the deficiency disease to which those vitamins are related

It may be argued that the minerals are an exception to this characterization and that they possess very decided and definite pharmacologic as well as toxic properties This is true, yet when considered in the light of the requirements for these minerals the amount necessary for pharmacologic or toxic action is truly enormous Iodine might be taken as an example Common doses of iodine when used as a drug are 20 to 30 grains a day, *36,000 times* the amount needed for the normal functions of iodine in the body Much the same thing is true of iron The reason we give large doses of iron in iron deficiency anemia is to secure adequate absorption and not because any such amounts are needed by the body

As has been remarked, this lack of toxic and pharmacologic action of the essential food substances, vitamins particularly, is relative, not absolute There is some suggestion

that a definite order in this regard, related to the function of the substance, may exist In general those food factors which seem to exhibit less special functions appear to be on the lower end of the scale of toxicity However, no investigations have been made of this interesting possibility

THE RELATION OF RESEARCH TO CLINICAL APPLICATIONS

The rapid increase in our knowledge of these accessory food factors and of the deficiency diseases to which they are related has stimulated much interest and enthusiasm for the clinical application of that knowledge At the same time it has been difficult to keep abreast of new developments and to transfer knowledge from the laboratory to clinical medicine, while a multiplicity of units, doses, preparations, names, and mixtures has been the source of much confusion As a result there has been an uncritical use of these accessory food factors, the vitamins particularly, as well as a failure to apply much of the knowledge which is available and applicable

A source of much of the confusion has been the inclusion of data and findings referable to animals in writings and discussions of nutritional disease in man Even with the best of intentions the inclusion of such material is apt to be very confusing to persons who are not specially trained in the field of nutrition A great deal of the current study and investigation of nutrition deals with animals and while some of it is transferable to man, much of it is not The inclusion of this material in clinical discussions almost invariably leads some to an uncritical and unwarranted assumption that the findings apply to humans The result is that false hopes and enthusiasm are aroused and useless and often expensive treatment is instituted which delays correct diagnosis and appropriate therapeutic measures

It should be possible, however, by a careful consideration of the known facts concerning nutrition and by a recognition of the potentialities as well as the limitations in this field, to utilize our new knowledge in a sane and effective manner in the practice of medicine To begin with, it seems clear that the function and action of these essential food factors is to maintain the health of the tissues and participate in the normal vital functions of the body Other than this they have, with certain exceptions little or no action All, then, we can reasonably hope to do with these substances is to relieve a deficiency if one exists, or to prevent its occurrence The use of these substances under other conditions or for other purposes is likely to be worthless and in general is contraindicated unless the results of critical study provide a justification, as in the case of some of the minerals Too often in the past these substances, vitamins in particular, have been used without any justification whatever and solely on the basis of wishful thinking

It may be argued in this connection that deficiencies of some essential factors are represented by clinical signs and symptoms (perhaps diseases) which as yet are not recognized as due to a deficiency and that by using a 'shot gun type of treatment such a relationship may be discovered This may be true, but as Szent Gyorgyi[1] has pointed out, it is unlikely that many additional, important essential food factors remain to be discovered or that important, hitherto unsuspected relationships between nutritional deficiencies and clinical syndromes will be disclosed Accessory food factors are detected mainly by the result of their deficiency, that is, by the corresponding deficiency disease Because of this the more important the substance the sooner and more easily will it be discovered The important vitamins A, B (complex), C and D, have been discovered, as has the dietary significance of iron, iodine, calcium et cetera Other possible factors, not yet discovered, are apt to be of relatively

much less importance For example, the recently discovered vitamin K is for many reasons less important than vitamin C

GAPS IN CLINICAL KNOWLEDGE

There are, however, two fields of study of the essential food factors in which little has been done although they may contain much which bears importantly on health and disease I refer to the problems of the optimum amounts of these substances and the interrelationships between them For the most part we have been concerned in the laboratory and in the clinic with minimum amounts of the accessory factors, i e, with amounts necessary to prevent the appearance of *disease* Almost nothing is known about *optimum* amounts and *their* effect on health Mere freedom from detectable disease is not enough Optimum amounts may be defined as those which would provide the utmost in health as far as nutrition is concerned These amounts cannot, however, as yet be expressed in a practical way in specific weights or units Furthermore, they will undoubtedly be greatly affected by interrelationships about which we know very little Why, for example, will one person develop scurvy and another beriberi on much the same diet? Just what is the nature of the B_1 sparing action of fat?

Other phases of this problem of nutrition are the influence of other disease on nutritive needs and the effect of nutritional deficiencies on associated disease Many deficiencies are, we know, conditioned, that is, they are induced by development of other disease without which they would not occur We have little exact knowledge of these effects, however In the opposite way nutritional deficiencies may affect other diseases, infections for instance Much has been written of this, but as one of us has pointed out, there is very very little reliable data with regard to these relationships Thus, although one cannot justify the

loose thinking and practice which characterizes much of the clinical work in nutrition, there are many opportunities for critical experimentation and observation in the very broad field of human nutrition

THE NATURE OF SUBCLINICAL DEFICIENCIES

Regardless of these considerations deficiency diseases are assuming increasing importance—partly because the control of other diseases such as infections brings them into greater prominence, but in a large measure because our increasing knowledge of them has enabled us to appreciate better their frequency and effects I do not refer to advanced or severe states of deficiency These are in fact becoming less frequent in this country and in many parts of the world On the other hand we are learning that the incidence of mild or subclinical deficiencies is much greater than had been thought Such mild, or latent, or subclinical deficiencies are of greater importance than the more advanced cases They are less obvious more difficult to detect, affect greater numbers of people and are more apt to pass unnoticed and hence untreated Their effects are often indirect, influencing general health and efficiency rather than producing characteristic disease syndromes

There is little need to discuss here in detail the cause of these deficiencies Sometimes they are due to poverty, often to ignorance or custom, or to a combination of these On the other hand they may be due to causes peculiar to the individual, most often the influence of some other disease If due to the former causes they are usually endemic, but they may occur in epidemics as in wars or famines, with crop failures, or in institutions jails, and the like Such endemics and epidemics are primarily public health problems Pellagra is a good example of an endemic deficiency

disease in this country but it is not the only one In fact there is reason to believe that most of the nutritional deficiencies are endemic to a greater or less degree in all parts of the country, more so in some parts than others, changing in incidence and type from time to time On the other hand, if due to causes peculiar to the individual they are sporadic and belong particularly to the province of the practicing physician The exact frequency of the various deficiencies is unknown or difficult to determine accurately for reasons which will be discussed later

It is clear that the first problem in practice is to determine when a deficiency exists or is likely to occur This is not altogether an easy matter While simple enough in the fully developed deficiencies, such as scurvy and pellagra, it is more difficult to diagnose latent or subclinical deficiencies These are, however, the cases which must be diagnosed if full advantage is to be taken of the newer developments in nutrition and progress is to be made in the diagnosis, prevention, and treatment of these diseases It is of no advantage to wait until the deficiency diseases are fully developed, and pellagra, scurvy, beriberi, et cetera are obvious, before a diagnosis is made and before treatment is begun

In general, there are four methods for diagnosing dietary deficiencies They are 1 *an analysis of the diet*, 2 *the signs* and symptoms of a deficiency as determined by history and physical examination, 3 laboratory tests, and 4 a therapeutic trial With few exceptions, no one of these alone is diagnostic in early cases There is, however, new knowledge of the clinical manifestations and the signs and symptoms of the deficiencies, as, for example, in riboflavin deficiency, and new and improved laboratory procedures for detecting still earlier evidences of the deficiencies are being devised By the careful use of all these procedures, it will be possible in many cases either to detect early deficiencies and institute proper treatment or disprove the presence of a deficiency

A characteristic feature of nutritional deficiencies in humans is their multiple nature Single deficiencies are uncommon, multiple deficiencies are the rule This is in sharp contrast to the laboratory where single deficiencies predominate by choice and are purposely produced singly when possible This is desirable experimentally and has been very valuable, but has undoubtedly been the explanation for delays uncertainty, and error in transferring laboratory findings to clinical medicine In the clinic the multiple nature of the deficiencies has favored a careless attitude toward diagnosis on the part of the physician The tendency has been to foster careless and incomplete diagnosis and the complacent acceptance of such general terms as deficiency disease ' multiple avitaminosis and the like This attitude will in turn lead to the uncritical use of complex mixtures in treatment Such a practice is no more justified than failure to diagnose other diseases accurately One might as consistently make a diagnosis of heart disease and give digitalis, glyceryl trinitrate, diuretics, and thyroid extract, without attempting to determine the cause, pathologic changes, and functional disturbance The only proper procedure in any case of suspected nutritional deficiency is to determine as completely as possible the nature of the deficiency and the pathologic and functional changes resulting from it In this way the clinician not only insures accurate, complete diagnosis and proper treatment but is able to contribute to our knowledge of the vitamins as they relate to human health

THE TREATMENT OF DEFICIENCIES

Having made the diagnosis of a nutritional deficiency the matter of treatment is relatively simple in the sense that the treatment is specific, its nature is known and the results highly successful if treatment can be given There are how

ever, certain difficulties, often more social and economic than medical, which must be overcome and certain abuses which must be considered

Treatment may be divided into preventive or prophylactic treatment and curative treatment Under preventive or prophylactic treatment I have preferred to designate a subclass, *protective* treatment, for reasons which will appear later Though a subclass it is an exceedingly important one, particularly in the practice of medicine

In perhaps no other group of diseases does *prevention* offer as great possibility for complete success as in the nutritional deficiencies *Preventive* treatment may be taken to mean general prevention, prevention in the population as a whole As such it is entirely a matter of diet alone, except under certain abnormal conditions as war or famine when supplements of concentrates or pure substances may be included This general prevention is a matter of economics and education, the availability of adequate food supplies at a proper cost to a population educated to a knowledge of a balanced adequate dietary Such prevention is properly a matter for public health agencies and the physician is less concerned with it than with other forms of prevention to be considered later Nevertheless, the physician must not forget his obligation to the public as a teacher and a leader and in particular to his clientele who look to him for information and guidance on matters of health as well as illness He should, therefore, be prepared to give sound current advice in matters of diet and nutrition in so far as this is available clinically, keeping abreast of new developments in a critical but progressive manner

Protective treatment is, as indicated, a subdivision of preventive treatment, but a very important one It is the prevention of deficiencies in persons who for any reason are especially or peculiarly exposed to the possibility of a deficiency It includes all of the cases of so called conditioned

deficiencies, the sporadic cases due to circumstances peculiar to the individual, and the deficiencies likely to be associated with certain physiologic strains or stresses Therefore, protective treatment applies to individuals or certain groups of individuals such as pregnant women children at certain ages, patients with certain diseases, such as gastrointestinal disease, mental disease, et cetera, the particular groups and individuals differing with respect to the different nutritional factors It is this group which is of particular interest and importance to the individual practicing physician and it is this group for which he is particularly and almost wholly responsible This responsibility is heightened by the fact that if it is properly met such deficiencies can be entirely prevented and the ill effects, which are apt to be more than usually severe and damaging, can be avoided In this *protection* or *protective* treatment diet alone may not be adequate and here concentrates and pure preparations find a proper place

Curative treatment is to a high degree specific, and successful, restoring the patient to normal even after long and severe deficiencies in most (but not all) cases It may however, present problems of dosage, administration, absorption and the like as well as possible economic and social difficulties, problems which are present with preventive and protective treatment as well In considering these problems it will be helpful to list certain general principles of treatment To begin with the more natural is the source of the nutritional factors, the better The reason for this is obvious We know a great deal about nutrition but we do not know all about it in particular we do not know what may be lacking if dependence is placed solely on chemically pure products to the exclusion of possible but unknown factors in foods Neither do we know much about the interrelationship of vitamins or their relation to other constituents in the diet On the other hand we do know that a

liberal, well diversified diet will supply all necessary food elements and maintain normal health as far as nutrition is concerned Therefore, the order of use should be natural foods first, then concentrates of food or food like substances, such as yeast, cod liver oil and the like, and lastly pure preparations This is particularly true in the case of preventive or protective treatment and in the later stages of curative treatment when pure preparations should be replaced by concentrates and natural food substances The fact, however, that food and certain concentrates contain more than one food factor must not be taken as a recommendation for artificial combinations of food factors, especially vitamins, the use of which is in general to be condemned Whenever possible, natural dietary sources, *even when it may be necessary to use concentrates or pure* products, should be used and emphasized This is important, not only for the value of the additional food elements but to teach the patient the need and nature of an adequate diet and to prepare him for the time when the diet alone will be used to maintain the cure and prevent *recurrences*

If emphasis on natural food sources and diet is important, particularly in respect to general prevention and in the period of recovery after curative treatment, it is equally important that when concentrates and pure preparations are used, especially in protection and cure, that they be given *in amounts and by methods which will assure* adequate treatment To secure adequate protection, to make up for deficits, and to raise the intake to a safe level, it may be and often is necessary to use pure products and concentrates sometimes in large amounts, and sometimes by parenteral or special methods of administration One must, however, beware of over dosage, not because of harm or danger which is almost non existent with these substances but to protect the patient s pocket book and to avoid waste generally, the

possibility of which is greatly enhanced by the relatively high cost of some concentrates and pure preparations

CONFUSING FACTORS IN VITAMIN THERAPY

This problem of adequate yet proper dosage and treatment has been made difficult by the confusion arising from a multiplicity of names, preparations, units, and dosage, the use of irrational and often uncertain mixtures and to some extent the lack of accurate knowledge regarding the nature, effects and requirements of various products This confusion has lead on the one hand to a great deal of over enthusiastic, uncritical prescribing with wasteful over dosing, or inadequate administration, and on the other hand to a distrust of all vitamin and related medication and a failure to make use of these valuable products This state of affairs has not been helped by the action of many manufacturers in providing innumerable preparations and mixtures, using non informative or too suggestive names, obscure units, with unwarranted claims based on deceptive evidence

The manufacturer is not altogether to blame, however, and the physician on his part has the responsibility of knowing the status of various preparations the nature and basis of the units employed, et cetera In addition to the report of current investigations which must always, of course, be considered critically until fully established, he has the aid of such agencies as the Council on Pharmacy and Chemistry of the American Medical Association from whose periodical reports he may obtain information concerning the status and use of new nutritional substances and preparations

Neither the dealer nor the physician moreover, are to be blamed for much of the confusion which has its source in units and measures of potency Most of this is inherent in the rapid advance in the field of nutrition Units and

measures of potency are usually established in the beginning on the basis of animal experimentation. They are apt to be expressed in terms of animal units based on certain functions in animals, not necessarily found in man. This does not mean that units and the products to which they refer should not be used in medical practice. Often such products are found to be very valuable, even life saving, long before their exact composition is known and before the dosage can be expressed in exact and absolute terms. Unfortunately, however, the difficulty and confusion are increased by the introduction and use of more than one unit for the same substance. This may be very troublesome and the units may be difficult to reconcile or equate. Also, errors may occur in defining or determining units, errors which are afterwards corrected only slowly and with difficulty. Finally, there is a tendency to retain units of an indirect sort long after exact or absolute units (weight) can be assigned. Sometimes it seems that this is done purposely with the object of concealing the strength or value of certain products. Lastly, there is the possibility of error, fortunately rare, because products fail to possess the qualities or potency which they are alleged to possess in commercial advertising.

It can be seen from this rather brief discussion that whenever possible a unit should be an absolute value such as weight or volume. The opportunity to use such units ordinarily depends on the isolation or synthesis of a substance in pure or relatively pure form. At present many of the essential food factors including the vitamins thiamin, nicotinic acid, riboflavin, ascorbic acid, alpha tocopherol (vitamin E), and the substituted naphthoquinone (vitamin K) can be prescribed and dispensed by weight (milligrams) and no longer is it necessary to use vague and uncertain units for these substances. In these matters the individual physician can, of course, do little directly but he can support

proper agencies such as the Council on Pharmacy and Chemistry of the American Medical Association in their efforts to establish proper standards and units and by his patronage encourage the manufacturers to adopt, as soon as possible, these proper units and standards

It may also be concluded that pure substances should ordinarily be given separately or at most in simple combination of one or two items Pure substances are ordinarily given for curative or protective purposes and to meet specific needs and indications This is best met by individual preparations administered for individual purposes It is difficult to adjust dosage in mixtures and their use easily degenerates into the old shot gun prescribing with all its faults including inadequate treatment waste and, more importantly, a loss of critical faculties and judgment on the part of the prescriber The truth is that many of the ingredients of mixtures are present in such small amounts that in the recommended doses the amounts are too small to be effective and in adequate individual amounts the quantities are excessive or impractical, the cost needlessly high There is often in these cases a waste of the other ingredients which are either not indicated or are not needed in such large amounts To the argument that such preparations are good for prevention if not for curative treatment it may be pointed out that in the amounts needed for prevention these factors are ordinarily better provided and at less expense in food or concentrates of food

It is apparent that what has just been said does not apply to concentrates of natural food stuffs or semi food stuffs such as fish oils, yeast, wheat germ et cetera The fact however that such concentrates, which may contain more than one nutritive substance, are recommended for curative uses must not be taken as a recommendation for mixtures of pure substances, the use of which is in general to be con

demned Neither is it good practice to add pure preparations to food or food like concentrates

Such considerations raise the whole question of the fortification of foods with accessory food factors such as vitamins and minerals, a problem which is assuming increasing importance as the practice is broadened A complete discussion of this problem cannot be given here but in general it may be stated that the fortification of foods with the various nutritive factors, foreign to the food in question, or in unnaturally large amounts is inadvisable On the other hand the restoration of substances removed from food during processing may, under some circumstances, be justifiable

When possible the essential food substances should be *given by mouth All the reasons why oral administration* should be preferred to parenteral apply here and there are certain others in addition In the case of several of the vitamins the excretion is very rapid when they are administered parenterally so rapid that in spite of a deficiency large amounts may be excreted in the urine unused and hence wasted Some, such as K, appear to act more rapidly by mouth than by other routes, and, though the latter may be necessary in some cases, the oral route is best With iron, the effective parenteral dose is very near the toxic dose and it is often impossible to give adequate amounts, other than by mouth, without producing untoward effects Parenteral *administration then is to be restricted to specific and* clearly indicated circumstances and purposes The principal ones are, of course, inability to administer by mouth or a disturbance in absorption from the gastrointestinal tract

Finally, in concluding this introduction let me emphasize the need for as careful attention to these principles and to the scientific practice of medicine in the field of nutrition and nutritional disease as in other fields of practice Thorough, painstaking, scientifically honest observation and

application of our knowledge of nutrition must be employed not only for the benefit of the patient but to advance our knowledge of the various food factors as they relate to man, the final test of their relation to human health and disease

REFERENCE

1 Szent Gyorgyi Albert V On Oxidation, Fermentation Vitamins Health and Disease The Abraham Flexner Lectures Series Number Six, Vanderbilt University, Baltimore Williams and Wilkins, 1939

2

Vitamin-A Deficiency

(Night Blindness (Hemeralopia), Xerosis, Xerophthalmia, Keratomalacia, Vitamin A deficiency dermatosis, Hypovitaminosis A)

HISTORY

DEFICIENCY of vitamin A is the cause of one form of night blindness or hemeralopia and of those diseases of the eye long known as xerosis, and xerophthalmia, or keratomalacia It also causes a specific dermatosis and changes in the epithelium of certain internal organs, and a mild, latent, or subclinical deficiency may exist, presenting no clearly recognizable clinical signs or symptoms but detectable by instrumental means or by histological examination post mortem

Night blindness, a lessened visual acuity in dim light, has been known for centuries It was recognized by the Egyptians who treated it by feeding liver, a rich source of the vitamin In more modern times it has been found among prisoners and inmates of institutions, in sailors, in soldiers and the civil population in times of war and during famines Within recent times it has been common in Labrador during the winter, the inhabitants practicing the ingenious custom of bandaging one eye during the day to preserve it for use during the night At the present time it is reported from China, India, Africa, and in a mild form, even in this country

Xerosis, xerophthalmia, and keratomalacia may in gen

eral be considered progressively severer stages of the same disease of the eye, the last named complicated by secondary infection and destructive changes All forms have been recognized as occurring in association with and under con ditions which produce night blindness In more recent times xerophthalmia and keratomalacia have been observed most often in infants and children

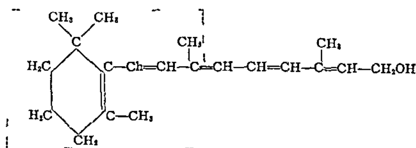
The specific relation of vitamin A deficiency to an erup tion of the skin was first reported by Frazier and Hu from China in 1930 Since then cases have been reported from other countries, particularly Africa and the Orient but also from England and the United States

Our knowledge of the pathologic changes in internal organs is mainly the result of the postmortem studies of Wolbach and his associates

NATURE AND FUNCTION

Vitamin A is a complex substance belonging to the group of the higher alcohols * Its chemical structure has a prac tical significance in that its alcoholic nature permits esterifi cation and combination with fatty acids, bile acids, and proteins This explains its combination with a protein to form visual purple (rhodopsin) its absorption as a bile

* Vitamin A is a high molecular primary aliphatic alcohol with the following formula

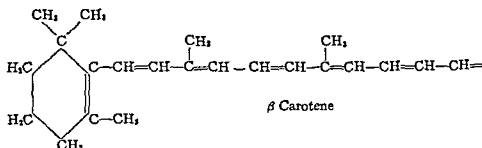


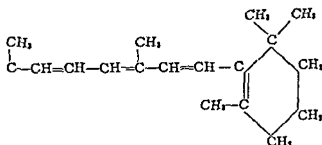
The optically inactive beta ionone ring (enclosed in the dotted lines) is an essential component of vitamin A structure Only carotenes with this ring are convertible into vitamin A Beta Carotene whose formula is

acid compound and its transport and storage in the liver as a fat acid ester or similar compound Its unsaturated bonds make it easily oxidizable and, since the highly oxi dized compounds have no biological activity, the practical importance of protecting the vitamin from oxidization in such procedures as storing, preserving, and processing food is apparent

Vitamin A is unique among the known vitamins in that it apparently is formed only by the metabolic activity of the *animal* organism from precursors or pro vitamins which are produced only by plants The precursors or provitamins of vitamin A are certain of the carotenes and carotenoids or pigments commonly found in plants Of the carotenes, four are important for man for conversion into vitamin A These are β carotene, α carotene, γ carotene, and cryptoxanthin Of these β carotene is the most important because it is more abundant in common plant foods of man and animals

given below has two of these rings and hence can be converted into two molecules of vitamin A





The unsaturated bonds in vitamin A are important in relation to oxida tion They are indicated by the double bonds

Under ordinary conditions man obtains vitamin A in part as the actual vitamin from animal tissues and products but the greater part is formed by converting the carotenes obtained in plant and animal food into vitamin A There is considerable difference among animal species in their ability to form vitamin A from the carotenes and some may not be able to do so Man possesses this ability but differences in the absorption and utilization of carotene as compared with preformed vitamin A are of practical importance in considering the supply of potential vitamin in the food or in medicaments The natural vitamin A has been isolated in pure crystalline form, but this product is not as yet available for general use The synthesis of vitamin A and isolation of the pure crystalline natural vitamin have been reported but such products are not yet available for clinical use

Vitamin A is absorbed from the intestine and transported in blood and lymph to the liver where by far the greatest part of it is stored Under normal conditions the absorption of vitamin A is very nearly complete for amounts near the minimum requirement though always there is some loss in the stool When larger amounts are ingested absorption is less efficient and with excessive amounts the greater part may be lost in the stool In states of depletion however, the absorption of large amounts may be more complete Bile is helpful in the absorption of A but is not necessary as in the case of carotene The absorption of carotene is subject to greater variations and factors which interfere with absorption affect carotene more than vitamin A itself Carotene also is carried to the liver which is probably the place where it is converted into vitamin A Injury to the liver may cause a decrease in the conversion of carotene to vitamin A and in such cases an increase in the carotene of the blood may occur in the absence of increased intake of carotene

With sufficient intake large reserves of vitamin A are

stored in the liver, sufficient to tide the subject over a considerable period of lowered intake This store tends to increase with age and the reserves of the infant are always lower than those of the adult irrespective of the mother s diet With a restricted intake the loss from the liver appears to be rapid at first but later the use is more economical When the store becomes low the circulating supply is inadequate and symptoms begin to appear In general the supply tends to determine the reserve level, a point of clinical importance because with a customary low intake the liver store is low and an acute shortage, caused by a sudden drop in intake or an increased demand, may precipitate the onset of symptoms, a thing which would not happen with a good reserve Infections and certain chronic diseases including diseases of the liver cause lower reserves by (1) decreasing intake, (2) increasing demand or (3) interfering with absorption and storage

Aside from the general growth effect vitamin A is essential for the formation of visual purple and for maintaining the health of the epithelial cells Except for its part in the synthesis of visual purple vitamin A, in contrast to some of the other vitamins, does not appear to participate in the activities of the cells, its relation to function being indirect through its effect on the health of the epithelial cells themselves

Vision in dim light is mainly a function of the rods of the retina and is dependent on an adequate supply of visual purple (rhodopsin) Visual purple is a compound of vitamin A and a protein In normal vision visual purple is broken down in bright light to vitamin A and the protein, part being destroyed In the dark visual purple is regenerated, in part from the previously broken down products and in part from new material An adequate supply of vitamin A is essential to the formation and regeneration of visual purple

An anti infective function has been ascribed to vitamin A There is little evidence, however, that it has any positive anti infective action Such action as it appears to have is probably non specific and related only to the maintenance of a healthy condition of the epithelium With regard to certain infections such as 'colds, even this ' favorable action ' has probably little effect on the *incidence* of infection though it may assist in shortening the infection and decreasing the frequency and duration of complications Even in the dermatosis due to vitamin A deficiency there seems to be little tendency to secondary infection when ordinary cleanliness is maintained

Certain other actions of vitamin A have been described These include an anti thyroxine action, a stimulating effect on epithelial growth in wound healing and anti anemic action, et cetera The existence of such properties is, however uncertain

PATHOLOGY AND PATHOGENESIS

The effect of a deficiency of vitamin A on the epithelium is an atrophy of the cells followed by replacement with undifferentiated epithelium through proliferation of the basal cells This results in a stratified, cornified epithelium, similar to the epidermis and the same in all structures irrespective of their original structure or function Disturbances in function result from this altered nature of the epithelium and the presence of masses of dead cornified cells Glandular structures such as the sebaceous and sweat glands diminish their secretion or cease entirely Ducts are plugged Specialized epithelial surfaces become replaced by a flat, keratinized surface

In the eye this process leads to a metaplasia of the epithelium of the conjunctiva and cornea with similar changes in the ducts of the lacrimal glands

The *skin* becomes dry and rough and there is hyper keratosis of the hair follicles, developing into a papular eruption about the pilosebaceous follicles (Fig 1) Hairs are broken off and lost In some cases the dryness and rough ness of the skin are less noticeable and the eruption resembles acne There may be a dirty slate gray pigmentation and in cured cases spots of pigmentation may remain about the area of the papule

Microscopically, the papule is seen to arise from the pilo sebaceous follicle and the lumen and mouth of the follicle are dilated and plugged by a dense mass of horny, cornified epithelium arranged in more or less concentric lamellae in which there is often a remnant of the hair There is hyper keratinization of the epithelium lining the follicle, hyper plasia of the adjacent epidermal cells and moderate hyper trophy of the horny layer The cutis vera around the follicles shows a mild grade of irritative inflammatory reaction with but slight cellular infiltration The sebaceous glands are absent or present only in remnants The sweat glands and their ducts show changes similar to those seen in the pilo sebaceous follicles Increase in the melanin pigment occurs in the skin and conjunctiva The acne like papules resemble microscopically those of acne vulgaris, except that cellular infiltration is much less The epithelium of the *respiratory tract* shows the keratinizing changes in all sites In the lungs the plugging of bronchioles by desquamated cells leads to areas of atelectasis and bronchiectasis, the cavities often filled with keratinized cells Interstitial or peribronchial pneumonia is a frequent complication in infants Similar keratinization of the epithelium is found in the *accessory sinuses, nares,* and *eustachian tubes* as well as in the *salivary glands*

In young children there is keratinization, desquamation, and replacement of the normal epithelium in the *kidney, pelvis, ureters, bladder,* and *uterus* Similar changes prob



FIG 1 A biopsy section of the skin from a case of vitamin A dermatosis Note the hyperkeratosis of the superficial epidermis The dilated hair follicle is filled with a plug of keratinized epithelium and there is hyperplasia of the epithelium about the follicle The sebaceous gland is absent and the sweat glands are decreased in number and size

ably occur in adults to a less degree and keratinization of the vaginal epithelium is seen in women Absence and defective formation of dentin and enamel, resulting in deformities of the *teeth* occur Changes in the ovaries and testes as well as other structures are recognized in animals but have not been established in man and there is as yet no conclusive evidence that deficiency of vitamin A causes specific changes in the central or peripheral nervous system

The resumption of a satisfactory supply of vitamin A is followed by repair which begins in a few days micro scopically but which requires a considerable time to be clinically apparent and complete except for the restoration of visual purple which takes place much more quickly In the process of epithelial repair the cells which have been irreparably damaged are cast off and the viable cells proceed to develop and differentiate into the specific type of epi thelium originally present in that site Complete restora tion does not occur in these places when secondary infection and destruction of tissue have been too extensive, as for example in the eyes

INCIDENCE AND EPIDEMIOLOGY

Severe states of vitamin A deficiency such as are repre sented by xerophthalmia severe dermatoses, and advanced night blindness are very rare in this country, especially in adults The literature in this country contains the report of only one case of xerophthalmia in an adult in several years and according to the experience of ophthalmologists it is very infrequent However, there is reason to believe that mild deficiency or hypovitaminosis A is fairly common among the general population, even in those apparently healthy, and particularly in younger children Recent re ports indicating the existence of mild deficiency among a

large percentage of children and even adults are open to question because of doubts concerning the accuracy and reliability of the methods used for detecting the deficiency Nevertheless the occurrence of the dermatosis, the detection of night blindness of sufficient severity to discount certain errors in diagnostic methods, the finding of gross and histologic changes at autopsy, especially in children, and an analysis of diets indicates that the condition is not uncommon A deficiency of vitamin A occurs among those who cannot or do not through ignorance, habit, or mistaken ideas, include a sufficient supply in the diet Among patients the incidence is considerably greater because of the greater likelihood of a "conditioned" deficiency and in practice it is found most often as a complication of other diseases It is apt to occur in infections, particularly chronic infections such as tuberculosis, in gastrointestinal disease and diarrheas, among those on therapeutic diets, in surgical patients, pregnant women and the insane The common practice of adding vitamin A to diets of infants has lessened its incidence among them Certain diseases of the liver which interfere with the conversion of carotene to vitamin A or the storage of the latter may result indirectly in vitamin A deficiency

SYMPTOMS AND SIGNS

The detectable symptoms and signs of vitamin A lack are those referable to vision, the structure of the eye, the skin and vagina Changes in the internal organs are diagnosed only by inference but may be detected at post mortem examination, especially in children

Night Blindness Idiopathic night blindness varies from mild states detectable only by instrumental means to a disability sufficient to make the subject blind in dim light

In the mildest forms the subject is unaware of the defect In the earlier clinical stages questioning may reveal the symptom or the patient may complain of it voluntarily Difficulty in reading or sewing at night, in finding a seat in a darkened hall, stumbling in the dark or trouble in driving an automobile at night are common complaints In driving an automobile the subject may be more sensitive to blindness by approaching head lights and may fail to see turns, road side obstructions and passing pedestrians clearly In the more advanced stages the symptoms are easily noted

Xerosis and Xerophthalmia These conditions are well known results of the changes in the epithelial tissues of the eye In the earliest stages (prexerosis) objective changes are slight or lacking and the principal manifestations are subjective, itching burning, asthenopia, and mild photo phobia (Similar symptoms are found in riboflavin defi ciency) Later there may be a slight conjunctivitis with redness of the lids The conjunctivae are, however, dry, tears are lessened and when the lids are held open for a few moments the conjunctival tissues and cornea become drier than normal With xerosis the conjunctiva become thrown into greasy folds, pigmentation and Bitot s spots appear and the symptoms are more severe This stage is fol lowed by involvement of the cornea which becomes ede matous and infiltrated with leucocytes with resulting opac ity Secondary infection is usually present by this time, the B xerosis being a common invader More serious infection may follow with ulceration and complete destruction of the eye Kruse has described the finer changes in detail (see Diagnosis)

Skin The specific dermatosis of vitamin A deficiency has been recognized only recently having been first described by Frazier and Hu from China in 1930 It consists of a papular eruption usually occurring first on the anterolateral

surface of the arms and thighs, spreading to involve the legs, abdomen, buttocks, back, and neck The lesions consist of dry papules of varying size up to 5 cm in diameter, arising at the site of the pilosebaceous follicles, principally on the extensor surfaces of the thighs and arms but extending in some cases to the shoulders, abdomen, back, buttocks and, rarely the face and neck The papules are conical or hemispherical and contain a central intrafollicular plug which projects from the surface or is covered with a loosely adherent scale When expressed the plugs leave gaping cavities In mild cases the papules are no larger than those of ordinary gooseflesh Washing may lessen the eruption considerably by mechanical effect The skin is generally dry, rough and wrinkled, darker than normal and often of a dull slate color There is an absence of sweating Comedones often are numerous

In some patients the lesions present a somewhat different appearance They consist solely of dull red, flat, or slightly conical, discrete papules of varying size, usually about 0 5 cm in diameter, similar to the lesion of acne The papules are distributed over the anterolateral aspect of the arms, the shoulders, upper chest and back, with few, if any, on the face, abdomen, or lower extremities The individual lesions often simulate a pustule, giving the impression that by piercing or removing the whitish top a bit of pus could be obtained When such attempts are made, however, the cap is found to be a thin, whitish scale which, when removed, leaves a raw surface and pus is not found In some cases there are a few scattered pustules but comedones are uncommon The skin does not appear dry or rough and does not itch

The dermatosis is said to be uncommon in children and Lowenthal related the increased incidence after puberty to the changes in the sebaceous glands and hair occurring at that time Recent studies suggest that mild grades of the eruption are not uncommon and will be found if looked for carefully

Vagina Changes in the vaginal epithelium, similar to those changes occurring in the skin, apparently may pro duce a vaginitis of the type which is commonly called senile vaginitis

Other Organs Alterations in the mucosa of the upper respiratory tract (nose and accessory sinuses) and upper gastro intestinal tract (mouth and salivary glands) produce few demonstrable effects Deformities in tooth structure and caries occur but the changes have no specific clinical characteristics The effects on the trachea and bronchi have been discussed in the section on pathology They seem to be pronounced and early in children but the clinical effects they produce are those of the complications, such as atelec tasis, bronchiectasis, and pneumonia which do not differ from the same conditions arising under different circum stances Still earlier effects of the atrophic process in the mucosa may occur but cannot be demonstrated as specific effects of the vitamin A deficiency Changes in other internal organs such as the pancreas genito urinary tract, uterus, et cetera, do not produce sufficiently characteristic signs or symptoms to establish recognizable clinical syndromes Al though excessive numbers of epithelial cells in the urine with the urinary tract as their source may represent the changes in the epithelium of the renal pelvis, ureters or bladder the evidence is as yet insufficient to establish this as reliable clinical evidence of vitamin A deficiency The evidence is still insufficient to relate vitamin A deficiency to the formation of renal calculi in man

The *complications* of vitamin A deficiency are usually not true complications but are diseases which are the pre disposing cause of the deficiency Secondary infection of the eye bronchitis and bronchopneumonia, and renal infections may be classed as complications Bronchopneumonia is the common cause of death in infants with vitamin A deficiency Secondary infections of the skin are not common if ordinary cleanliness is maintained

DIAGNOSIS

The diagnosis of vitamin A deficiency is made from a knowledge of a probably deficient intake or absorption and recognition of the changes produced in the various structures and functions affected In the severe deficiencies this is easy from the clinical signs and symptoms but such cases are rare In the milder cases slight or inconspicuous signs and symptoms must be checked by instrumental or laboratory methods or by the results of treatment

Analysis of the diet can be made from available food tables A careful record of the food consumed should be made over a period of several days, preferably two weeks For recording the diet use may be made of check diet lists or the subject may record carefully the food taken Children and others incapable of recording the diet should have it recorded for them by a reliable person Careful attention should be paid to the *quantity* eaten as well as to the various *items* A patient may say he drinks milk and he may—a glass or two a week Methods of storage and preparations should be scrutinized and possible difficulties in absorption taken into account

Inquiry should be made about the early symptoms of night blindness, dryness of the eyes, burning, redness, conjunctivitis, photophobia, and asthenopia Vaginal discharge and irritation may be present and physical examination reveal the early changes in the eyes and skin (Fig 2) All of these signs and symptoms are, of course, suggestive only but should lead to a consideration of the possible deficiency In some cases knowledge of the diet will lead to careful observation of slight signs In others, detection of the latter will cause a careful scrutiny of the diet A therapeutic test is helpful

The laboratory tests and special procedures used for the diagnosis of vitamin A deficiency are the test of dark adapta

tion with the photometer or adaptometer (night blindness), and determination of the concentration of vitamin A and carotene in the blood In addition Kruse has recommended the detection of changes in the conjunctivae with a slit lamp (biomicroscopy)

The principles and general application of the adap tometer test are as follows * The amount of light required for perception by the subject is measured, usually in ordi nary room light The visual purple is then bleached by exposure to a light of known intensity for a given time Following this the amount (intensity) of light necessary for perception is again measured Those with normal dark adaptation (adequate regeneration of the visual purple) require less light and a shorter time to return to normal after bleaching than deficient subjects, a difference which can be measured quantitatively

A number of instruments have been devised for this purpose but as yet no single procedure has received unqualified acceptance for general use Various sources of error exist in both instruments and the subjective response of the patient Vitamin A deficiency is not the sole cause of night blindness Nevertheless, when properly made such tests are reasonably reliable as clinical tests go particularly when repeated and supported by critical judgment of the effect of treatment

Kruse recommends the detection of changes in the con junctivae seen with the microscope under slit lamp illumina tion According to him they are the early changes of xerosis and are characteristic of vitamin A deficiency They consist of a loss of transparency in the superficial and deep layers, diffusely or in local areas forming spots, or plaques, often elevated and opaque, and with pigmentation Many stages of development are observed up to those grossly visible and

* Detailed directions for the various diagnostic tests are given in the appendix

the fully developed Bitot's spots. Additional changes in the surface of the conjunctivae, the vascular network and at times in the cornea itself, are observed. Although such changes undoubtedly occur in vitamin A deficiency, the specificity of these lesions has been questioned and for the present must be considered as suggestive only, to be checked by continued observation and therapeutic trial.

The concentration of vitamin A and carotene in the blood can be determined chemically with reasonable accuracy and reliability by techniques which are not too difficult. Although the concentration is subject to fluctuations not related to the general state of vitamin A nutrition, when precaution is taken to allow for such factors as meals, etc., the concentration may be taken as an index of vitamin A nutrition, subject of course to critical interpretation. Normal values by the method commonly used and described in the appendix are usually taken as 70 International Units per 100 cc. or above 45 for infants. Because of slower response of morphologic lesions to treatment and the quicker fluctuations of the blood values, very close correspondence between blood levels and other evidence of vitamin A deficiency is not always to be expected.

TREATMENT

The treatment of vitamin A deficiency states is specific and consists of the administration of adequate amounts of the vitamin under conditions which insure its absorption. Therefore discussion of treatment is concerned mainly with dosage, preparations to be used, absorption, methods of administration, duration, and possible ill effects of treatment. Pure vitamin A has been prepared from natural sources but is not available for general use. In practice, cod and other fish liver oils, concentrates of these oils, and carotene (pro



Fig 2 Dry papular lesions some reddened and some gooseflesh in vitamin A deficiency Before and after treatment with vita

vitamin A) are used U S P XII cod liver oil contains not less than 850 international units* per gram (about 15 drops) but most of the preparations in use today are con siderably more potent, many containing 2000 units per gram (about 8,000 per teaspoonful) and are really con centrates Twelve to 24 cc a day (3 to 6 teaspoonfuls) of these will therefore provide 24,000 to 48,000 units Still more concentrated preparations of cod liver oil and other fish liver oils are available, some of them in dry (tablet) form

The added food value of the oil and its relative cheapness make cod liver oil the preparation of choice in many cases However, some patients object to it Also, when it is advis able to give large doses the greater volume makes its use undesirable In these cases any one of the more concentrated preparations may be used In the case of those who refuse to take the cod liver oil, or in the case of children and infants when loss by spilling, breakage, et cetera, is apt to occur, the use of the more concentrated preparations may secure a surer intake and actually be cheaper Some con centrates also have the advantage of being suitable for mix ing with food, such as milk or milk formulas

All of the ordinary preparations of vitamin A (but not carotene) contain some vitamin D but some of them contain so little that it does not interfere with their use even as a therapeutic test except in very precise experiments Many preparations contain other vitamins and even minerals

Carotene or provitamin A possesses certain of the advan tages listed above and in addition can be given parenterally in those occasional cases in which oral administration is difficult or impossible or absorption by way of the gastro intestinal tract is prevented Approximately double the dose (in units) should be given when carotene is used and it

* The international unit is the activity of 0 6 microgram of pure beta carotene

should be remembered that factors which interfere with absorption affect carotene to a greater degree than vitamin A itself This is important in the treatment of vitamin A deficiency in contrast to prevention because the very factors which require a large intake and rapid storage of the vitamin often interfere with the absorption of carotene

Whenever possible the giving of vitamin A should be accompanied by a diet rich in vitamin A containing foods, not so much for the value of the additional vitamin but to teach the patient the need and nature of an adequate diet and to prepare him for the time when the diet will be depended upon to maintain the cure and prevent recurrences of the disease Due consideration must naturally be given to other requirements of diet

Prevention is as important as treatment Although the optimum intake under varying conditions of age, sex, and physiologic needs remains to be determined, the recommended allowances of the Food and Nutrition Board constitute safe daily intakes These are 5,000 international units for an adult, man or woman, 6,000 and 8,000 during pregnancy and lactation, and 1,500 to 4 500 units for children under one year to twelve, with older children 5,000, and 6,000 for boys 16 to 20 Larger amounts should be provided as a prophylactic for patients liable to develop the deficiency, particularly if the likelihood of the deficiency is due to an interference with absorption Ordinarily 3 000 to 5,000 units can be obtained from the diet, and for many reasons this source is to be preferred, but it must be remembered that much of vitamin A in the diet comes from carotene, for which double the requirement should be allowed For those individuals needing special protection, such as some children and pregnant or lactating women, it is best to add a supplement

Although many mild states of vitamin A deficiency may be cured by dietary intake alone the presence of any of the

clinical syndromes described probably constitutes an indication for the giving of additional vitamin A This should always be done when difficulties in absorption exist or are suspected, or when there is any doubt regarding the intake of adequate amounts in the food This is often a factor when the deficiency occurs as a complication of other disease

Daily doses of 20 000 to 40 000 international units of vitamin A or equivalent amounts of carotene should be ample in any ordinary uncomplicated case of vitamin A deficiency This is approximately the amount contained in 32 to 64 cc (two to four tablespoonfuls) of cod liver oil meeting the *minimal* U S P XII requirements (850 international units per gram) Much smaller amounts cure mild cases of the deficiency slowly but a liberal excess should be provided for safety Some loss always occurs in the intestines but the doses given above should compensate for the loss It is very doubtful whether any advantage is gained in even the speed of recovery by the use of amounts larger than 40 000 to 50,000 units except perhaps in the case of night blindness However, in rare cases of severe deficiency such as xerophthalmia it may be advisable to give larger doses with the idea of preventing any possible delay in repair which might allow permanent damage to occur Larger doses should be used when there is difficulty in absorption or in patients with liver damage which may interfere with storage A great and rapid improvement in night blindness in cirrhosis of the liver when the dose of vitamin A was increased to 40 000 or 50,000 units has been reported

With adequate doses and satisfactory absorption night blindness responds to treatment rapidly An improvement has been noticed within a few hours but doubt of the accuracy of the method employed for testing the power of dark adaptation makes this observation somewhat unreliable In most cases a response can be observed in a few days and recovery in mild cases should occur in two to four weeks

It is possible that in the case of night blindness recovery can be hastened by the use of larger doses

Xerosis also responds quickly but xerophthalmia, the dermatoses, and probably the more severe changes in the lungs and other internal organs, often require a longer period Dark adaptation probably requires only a supply of the vitamin sufficient for the formation of visual purple Changes in the epithelium, requiring a process of anatomical repair, respond more slowly Restoration of normal vision should not be taken as an index of complete repair of epithelial structures, a caution which is supported by the observance of relapses in some cases of the dermatoses The latter often require ten to twelve weeks to disappear It may be doubted whether excessive doses are more effective or more rapid in action in the cure of the epithelial lesions than smaller doses provided reasonable amounts are given and absorption is normal Simpson and Mason have reported cure of the vaginitis in one to four months, three fourths of the subjects being free of symptoms in two months

In keratomalacia moist compresses and protective dressings should be used locally in addition to the specific treatment Recovery from this severe manifestation may be accompanied by extensive scarring and permanent disability Secondary infections of the skin, which are not common, are usually adequately treated by simple cleanliness, *the usual care given such infections*, and the specific therapy

Foods relatively rich in vitamin A or its precursors, the carotenes, are apricots, fresh or dried, green asparagus, green beans, broccoli, butter, carrots, chard, cheese, collards, dandelion greens, eggs, kale, green lettuce, liver, milk, parsley, peas, peppers, prunes, spinach, squash, sweet potatoes, tomatoes, and turnip greens

Because of the many factors involved recommendations of specific diets are impossible here nor is it advisable to

foster excessive vitamin consciousness in the public as far as the every day consumption of food is concerned In general a diet affording as it should liberal quantities of green and yellow vegetables, milk and milk products, and eggs can be depended upon to supply adequate protection for normal subjects Larger amounts of milk should be given to children It must be remembered, however, that variation in the content of vitamin A and carotene of food occurs in different samples and under different conditions of seasons, feeding storage, and preparation and that the quantitative factor in food intake is one which is important and often neglected For more accurate determination of the vitamin values of foods, particularly for use in diagnosis reference should be made to tables of vitamin values of foods and an analysis of the average diet consumed over a reasonable length of time

Interferences with absorption constitute one of the few important difficulties in the treatment of vitamin A deficiency states The amount and form in which vitamin A and carotene are given, whether in the food or otherwise, the kind of diet, the presence or absence of bile from the intestine the need of the body for the vitamin and the degree of saturation all affect absorption Lessened absorption occurs in infections, diarrheal states ulcerative lesions of the gastrointestinal tract and when bile is excluded from the intestine (in the case of carotene) Injury to the liver *may not only interfere with absorption but may lessen or* prevent storage Low fat diets decrease absorption as do steatorrhea, celiac disease, and pancreatic dysfunction Ordinarily mineral oil interferes with the absorption of carotene but not of vitamin A

It is unlikely that injurious effects result from even excessive amounts of vitamin A itself In animals various toxic effects including cachexia, conjunctivitis, diarrhea loss of weight, anemia, degenerative changes in the paren

chymatous organs and muscle, and fatty infiltrations have been described But grossly excessive doses have been used and the possibility that the ill effects have been due to toxic substances contained in the oil has not been disproved None of the usual preparations contain sufficient vitamin D to cause the toxic effects of that vitamin Occasionally mild symptoms are seen at the beginning of treatment These consist of a slight exacerbation of the symptoms of the dis ease with a mild malaise which disappears in a few days A few persons are found to be allergic to the fish oils Hyper carotenemia resulting from the ingestion of large amounts of vegetables containing carotene may cause a yellow dis coloration of the skin (xanthosis cutis) but no ill effects result

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3

Thiamin (Vitamin-B$_1$) Deficiency

(Beriberi—Certain forms of peripheral polyneuritis—Hypovitaminosis B_1, et cetera)

HISTORY

THE classical result of B_1 deficiency is beriberi, a disease characterized by multiple neuritis, muscular atrophy, cardio vascular changes, and in many cases a massive edema These, however, are the result of a severe deficiency and mild de ficiency or hypovitaminosis B_1, which is much more common, presents a much less characteristic clinical picture with vague neuritic pains, anorexia, digestive disturbances, and less severe cardiovascular manifestations The fact that single vitamin deficiencies rarely occur in practice makes it difficult to determine the exact part played by vitamin B_1 in some of the symptom complexes occurring in patients apparently mildly deficient in this vitamin Also the effect of a slight deficiency complicating other disease, particularly other diseases of the heart and circulation and gastrointes tinal tract is not fully understood

Beriberi has been a problem for centuries, particularly in those countries of the Far East, Japan, China India, the Philippines, and Malay Peninsula, where its incidence in epidemic and endemic proportions has made it a socio eco nomic problem of great importance Conversely, socio eco nomic problems poverty and ignorance, famine, et cetera, have been responsible for the high incidence of the disease

References to it in occidental literature have occurred

for centuries The first occidental physician to describe it was Bontius in 1642 Known also as kakhe, asjike, loempe, and by such descriptive terms as endemic multiple neuritis, panneuritis endemica and polyneuritis endemica, it has in the past probably included, or been confused with, other diseases such as nutritional edema and scurvy Likewise special names such as ship beriberi and infantile beriberi have been given The latter, known as 'taou,' or 'suba disease, was not recognized as beriberi in the Philippines until 1904 Nevertheless, the disease in the countries where it is common presents a well defined and consistent clinical picture This is not so true of the sporadic and particularly the mild, subclinical cases, or hypovitaminosis B_1

It is scarcely an exaggeration to say that the disturbing effects of beriberi in the Japanese Navy and the efforts of the medical staff to control the disease led to the modern attack on the problem and initiated the series of studies which have led finally to our present knowledge of the dis ease Studies in the Japanese Navy gave firm support to the theory of a dietary deficiency as the cause It was Funk s efforts to isolate the antineuritic factor associated with beri beri which gave us the name 'vitamine and started the long series of researches which led to the isolation of thiamin (B_1) by Jansen and Donath (1921 27) and its synthesis by Williams and Cline (1936)

NATURE AND FUNCTION

Vitamin B_1 is thiamin,* a pyrimidine thiazole compound which has been isolated and synthesized in pure crystalline form In practice it is used in the form of the hydrochloride † It is a member of the so called B complex, a group of vitamins closely associated in nature, and was the first to be clearly differentiated from other members of the group

* Known also as aneurin water soluble vitamin B vitamin B *antineuritic* vitamin antiberiberi vitamin et cetera.

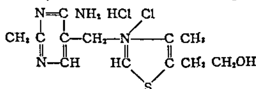
Undoubtedly this was due in a large part to its sensitivity to heat which enabled investigators to distinguish it from other members of the B complex Like other members of this group it is water soluble It is easily destroyed in neutral or alkaline mediums, much more resistant in acid mediums Heat is destructive in neutral or alkaline solution but pure solutions of thiamin chloride hydrochloride can be sterilized without loss at 120°C for thirty minutes because of the protective acidity of this salt

Vitamin B_1 plays an important role in the fundamental processes of oxidation in the body and is active as a coenzyme (cocarboxylase) in the oxidation of carbohydrate When thiamin is deficient pyruvic acid accumulates in the tissues Pyruvic acid is a normal intermediary product of carbohydrate metabolism which under normal conditions is removed as rapidly as formed It is possible that other metabolites may accumulate in abnormal amounts or that abnormal metabolites may be formed As will be seen the accumulation of pyruvic acid can be demonstrated and used clinically to study thiamin deficiency In its relation to carbohydrate metabolism it is significant that the need for thiamin appears to depend in part on energy production and to be influenced by the proportion of calories furnished by carbohydrates Fat possesses a sparing action on thiamin requirements, the mechanism of this is not altogether understood but may be due in part to the supplanting of carbohydrates by fat in heat production

In spite, however, of this knowledge of the chemical prop

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† Thiamin chloride hydrochloride has the following structural formula



In the body it is phosphorylated to form the pyrophosphoric acid ester which is active as a coenzyme (cocarboxylase) in the oxidation of carbohydrates

erties and action of this vitamin and its intimate relation to oxidizing processes, little is known of the mechanism by which a deficiency of it produces the physiologic and patho logic changes which occur in patients or in experimental animals At present it seems unlikely that they are due to the accumulation of metabolites, rather that the interfer ence with oxidation deprives the cell of normal nutrition In man as well as in experimental animals B_1 seems necessary for the maintenance of the health of nerve tissue, appetite, normal intestinal and cardiovascular function as well as normal growth and development in the young The latter, which is a property common to all vitamins, may be con sidered a non specific function It is probable, however, that its action and significance is much wider than indicated above Its action is not confined to man or animals Thiamin plays a fundamental part in the physiology of plants, seeds, and micro organisms, as well as animals and is probably part of the fundamental mechanism of most living things

Thiamin is widely distributed in the body but, accord ing to *animal experiments* is particularly abundant in the liver, heart, kidneys, and voluntary muscle The brain con tains about one third as much as muscle It has been sug gested that the liver stores the vitamin These variations in content may be significant in relation to the development of pathologic lesions and the signs and symptoms of a de ficiency Granted that there is particular need for the vita min in muscle the need for a larger amount in such a mus cular organ as the heart and a particular sensitiveness of the latter to a deficiency is conceivable The characteristic in volvement of the heart in acute and fulminating beriberi is interesting in this connection

Thiamin is present in the blood, both cells and serum, in small amounts which can, however, be measured quantita tively by appropriate methods It is excreted in the urine in amounts which reflect intake and storage and this excretion forms the basis for clinical tests of vitamin B_1 deficiency

(See Diagnosis) There is also some evidence that in addition to thiamin itself, break down products of thiamin also are excreted in this manner

The vitamin is readily absorbed from both the small and large intestine and it is unlikely that much difficulty in ab sorption occurs except under extreme conditions In normal human subjects there is a small but fairly constant amount of B_1 in the feces Part of this may represent that synthesized by bacteria but it is unlikely that such thiamin is usable as animal experiments indicate that it is necessary to consume feces by mouth in order to get the synthesized vitamin There is some evidence to indicate that a considerable loss may occur in the stool when large doses of the pure vitamin are administered by mouth Such administration, parenteral as well as oral, always produces a large excretion in the urine, except perhaps when only moderate sized doses are given for a short time to very deficient subjects

Milk contains the vitamin in amounts which reflect the maternal intake Animal studies indicate that amounts ade quate for the young require a considerably larger intake by the mother than that necessary for the protection of the mother herself This is significant in relation to the occur rence of infantile beriberi and perhaps even more so in respect to suboptimal growth of infants in cases of mild or subclinical deficiency

Thiamin has no pharmacologic action in the ordinary sense that is, it is without established effect in deficient subjects

PATHOLOGY AND PATHOGENESIS

In considering the effect of a lack of thiamin the earliest changes are those in function the *pathologic physiology* of the disease With the limited storage capacity for B_1 in the human organism evidences of disturbed function appear quickly if the intake is reduced to a very low level Studies

on human subjects indicate that some symptoms appear in as short a time as ten days Under natural conditions the deficiency is usually not so complete and the onset is slower Symptoms indicating a disturbed physiology, if not structural pathologic lesions, are limited growth in children, anorexia and vague indigestion," muscle soreness and "neuritic pain, fatigability and the symptoms of neurasthenia None of these is specific but their occurrence on deficient intakes, progression with continuing deficiency and relief when thiamin is given indicate that they represent disturbances in function due to such a deficiency With these symptoms there is a decreased excretion of thiamin in the urine and under certain circumstances an increase in pyruvic acid in the blood In the earlier and milder cases this disturbance in the oxidation of pyruvic acid and its accumulation is not demonstrable except following a period of muscular exercise or the ingestion of glucose This places an increased load on the oxidation mechanism, which with a shortage of B_1 causes an abnormal accumulation of pyruvic acid Thus even in early deficiency a chemical "lesion" can be demonstrated Just how the chemical lesion is related to the production of morphologic lesions and symptoms is not known It now seems unlikely that it is a result of the direct toxic action of pyruvic or any similar metabolite More likely it is due to a failure of proper energy supply to cells

The lack of specificity of the pathologic changes in beriberi or B_1 deficiency is indicated by McLaughlin and Andrews criteria[1] for beriberi on gross examination (1) dilatation and hypertrophy of the right side of the heart, (2) congestion of the viscera (3) anasarca, and (4) absence of other findings to account for death Microscopic changes though clearly evident in sufficiently developed cases show a similar lack of specificity

The heart and the peripheral nerves exhibit the most constant and important lesions and the degree of involvement of them varies with the type of the disease In the *wet*

group and particularly in the *acute* cases the outstanding feature aside from the anasarca, is the appearance of the heart It is greatly enlarged, the enlargement being more on the right side and more in the auricle than the ventricle The right auricle is often greatly distended, the wall very thin, while the right ventricle though dilated has an in creased thickness of the wall which is often greater than the left The cavity is enlarged, the trabeculae and papillary muscles are prominent but the valves are normal There is a dilatation of the conus arteriosus, which Wenckebach[2] thinks is peculiar to this disease Microscopically either little may be seen or there is degeneration and what some have interpreted as edema of the muscle fibers

The edema visible externally is matched by a hydro thorax hydropericardium, and ascites as well as edema of organs and tissues In chronic cases there is a chronic passive congestion of the viscera, the liver in particular being nearly always enlarged with congestion, and there may be pul monary edema Microscopically there is some cloudy swell ing, or fatty degeneration

The nature of neither the enlargement of the heart nor the edema fluid is certain A neuritis of the vagi fails to explain the difference in the two sides of the heart and changes in the heart do not parallel changes in the nerves The enlargement does not seem to be a true hypertrophy and disappears with astonishing rapidity under adequate treatment It has been suggested that edema is the cause of the enlargement but this has been objected to on the basis of the selective localization of the enlargement Such a selective localization, however, possibly may be based on a variation in thiamin requirements or greater sensitization of certain parts to a lack of it Such a condition or state of affairs is suggested by experimental studies of the accumu lation of pyruvic acid in different sites in different animals the concentration of this substance varying in different tissues and organs Furthermore, it has been shown in ani

mal experiments that there is a difference in the uptake of oxygen in different parts of the heart under conditions of B_1 deficiency Also, the changes are not confined to the heart and it may be that it, by a greater sensitivity, merely shows the changes oftener and earlier which would account for the apparent selectivity

The changes in the heart are related to the nature and mode of formation of the edema fluid The simplest apparent explanation is that the edema is that of congestive failure This is supported by the changes in the heart, by the evidence of elevated venous (capillary) pressure and by the chronic passive congestion of various organs It is also consistent with the nature of the edema fluid which seems to be a simple transudate Most of the cases of true wet beriberi occur in the Orient under conditions of diet which suggest a protein deficiency and a hypoproteinemia as at least a contributory factor These more simple explanations for the cause of the edema have been objected to, however, and it has been attributed to a specific effect of the vitamin Just how this acts is not explained The easiest explanation, that of increased capillary permeability, is opposed by the nature of the edema fluid which in the few cases in which it has been determined, has not shown the content of protein which would be expected if this were the mechanism Yet the careful study of one or two cases in which the edema did respond directly to the administration of pure thiamin, makes such a relation possible More thorough study of characteristic cases with modern methods is necessary to explain the mechanism Sporadic cases seen in this country, often in association with other diseases, are not good material for such studies

The lesions of the peripheral nerves are found predominantly in the dry (usually chronic) type but in the so called mixed forms as well They are not apparent grossly and consist of a panneuritis beginning with a degeneration of the myelin sheath, later including fragmentation of the axis

cylinder and wallerian degeneration The various changes especially the earlier lesions, are shown best by the special stains The nerves in the legs are ordinarily affected first and in all nerves the process begins in the distal portion Not all the fibers are involved, the number increasing with the severity and increasing duration of the disease In addition to the nerves to the extremities the cranial nerves, particularly the vagi and phrenic, and those of the trunk are affected Lesions have also been described in the sympathetic fibers

The central nervous system is little affected though degeneration of the sheaths of scattered fibers, especially in the posterior columns, and anterior and posterior nerve roots are observed together with changes in some of the cell bodies in the ganglia, anterior horn cells, and in the medulla

None of the changes in the nerve tissue are specific Indeed, some observers deny that they are the result of B_1 deficiency Recent animal studies, besides casting doubt on the specificity of thiamin in relation to neuritis, implicate others of the B group, notably pyridoxine Also there is a tendency to separate etiologically the cardiovascular and peripheral nerve manifestations of what has been considered thiamin deficiency Nevertheless, for the present, the changes described may be considered clinically the effect of thiamin lack Muscles supplied by the affected nerves show on microscopic examination atrophy with loss of striations and cloudy swelling or fatty degeneration They are not however, specific for beriberi or vitamin B_1 deficiency and are found in other forms of neuritis Besides these changes and those in the heart and nervous system other lesions such as hypertrophy of the islands of Langerhans, enlargement of the adrenals (medullary) thyroid and hypophysis have been reported but as yet nothing certain is known of the possible relation of such changes to the deficiency of vitamin B_1

These apparent, if non specific, lesions are those of beriberi or moderate to severe vitamin B_1 deficiency Unfor

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tunately, little or nothing is known of the pathologic changes in deficiencies which, while mild, yet are sufficient to produce symptoms It is not known whether the neuritic pains and tenderness of muscles in these mild cases are accompanied by demonstrable lesions in the nerves or whether the milder cardiovascular symptoms have a background of demonstrable lesions in the heart It is, perhaps, not to be expected that the earlier and milder stages of the deficiency will be accompanied by morphologic lesions, rather that the search for the lesion in this early stage must be in the field of function

INCIDENCE AND EPIDEMIOLOGY

Beriberi is endemic in the Far East, where it assumes epi demic proportions from time to time In the United States there is said to be a small area of endemic beriberi in the rice growing sections of Louisiana Occasional, small epi demics in jails and institutions occur in this country and in Europe but only rarely, in times of war or famine, does it appear in epidemic form in the Western World Here by far the greatest incidence is in the form of sporadic cases, in persons who, for some reason peculiar to the individuals, fail to ingest or absorb an adequate amount of the vitamin The common reasons for this failure are alcoholism, gastro intestinal disease, psychoses and psychoneuroses, therapeutic diets, dietary faddism, ignorance, and poverty Some cases of polyneuritis associated with infectious disease are probably due to this cause and the polyneuritis of pregnancy is most likely a neuritis due to B_1 deficiency Neuritis due to lack of B_1 is a common associate of other deficiency diseases and is frequently seen with pellagra

If only cases of manifest deficiency or beriberi are con sidered the incidence of the disease in this country is very low It is probable, however, that in the milder grades it is very common Numerous cases exhibiting the cardiovas

cular manifestations of the disease have been reported from New York and other large cities by Weiss and others,[3] and neuritic manifestations are frequently seen in pellagrins in the South Cases of mild alcoholic neuritis are fairly common and if patients with mild neuritic pains anorexia, and gastrointestinal disturbances which *appear to respond to treatment* are accepted as instances of the deficiency the disease is relatively frequent in ordinary practice However in the current enthusiasm for vitamins an uncritical atti tude toward diagnosis fostered by the unrestrained promo tion of some manufacturers has developed and in the ab sence of specific and simple clinical tests for mild states of the deficiency, little reliance can be placed on this evidence Even other deficiencies, such as calcium lack, may produce similar symptoms Often no attempt is made to use such diagnostic aids as are available, as for example a careful analysis of the diet Nevertheless mild deficiencies are more common than has been thought in the past and should be watched for, particularly in patients who for some reason are particularly subject to a possibly deficient intake or diffi culty in absorption of the vitamin

In areas of endemic beriberi the disease is common in infants and children as well as in adults but in this country it appears to be less frequent in children This is probably because of the greater number of conditioned cases, a type which is less frequent in children However, it is pos sible that a *slight* deficiency in children expresses itself as a detriment to optimal growth though other effects of the deficiency are not manifested

SYMPTOMS AND SIGNS

Classical beriberi occurs in two primary forms the so called *wet* and *dry* The former is characterized by edema and cardiovascular symptoms and signs the latter by pe ripheral neuritis and muscle atrophy However, the neuritic

manifestations often occur with the wet form, the "mixed cases Beriberi may appear abruptly—acute beriberi This is most often the wet form in which the patient becomes suddenly and grossly edematous It may develop more slowly, as subacute beriberi, or very gradually, as chronic beriberi In wet beriberi, appearing suddenly or gradually, edema is the outstanding symptom, often masking a loss of weight, which accompanies and often precedes it

In chronic beriberi in adults the onset is gradual with a prodromal period marked by the vague and ill defined signs which constitute the manifestations of mild deficiency or hypovitaminosis, loss of weight and strength, fatigue, heaviness and stiffness of the legs, soreness of the muscles, neuritic pains, loss of appetite, headache, insomnia, dizziness, and indigestion After a variable length of time depending upon the degree of deficiency or the occurrence of precipitating factors the major symptoms and signs develop In the wet form the most prominent is edema, often amounting to anasarca with pleural, pericardial, and peritoneal effusions The edema commonly begins and is most severe in the legs, being influenced by gravity With this there is a variable amount of palpitation shortness of breath, tachycardia, and cyanosis, the signs of congestive failure There may be precordial pain Examination discovers an enlargement of the heart, usually greatest on the right, murmurs of the type associated with enlargement, a rapid, thready pulse, increased venous pressure and signs of pulmonary congestion There may be temporary elevation of the systolic blood pressure with increased pulse pressure, a full, poorly sustained pulse and a pistol shot sound over peripheral arteries Electrocardiographic tracings show changes consisting of deviation of the RS T segment, lengthening of the Q T interval and diminution or slight inversion of the T waves in any of the leads These changes are not specific but are very suggestive in a proper setting and particularly if promptly and completely abolished by adequate treat

ment with thiamin Although the enlargement of the heart is usually right sided, cases of the sporadic type may show enlargement of both ventricles and a mixed rather than a pure right sided type of failure Also cardiac changes due to B_1 deficiency complicate other forms of heart disease Pa tients with chronic and mild cardiovascular symptoms or even those with the neuritic type of beriberi may at any time suddenly develop alarming symptoms of cardiovascular collapse leading to death, with pulmonary edema, severe dyspnea and cyanosis enlarged liver, increased peripheral edema falling blood pressure, and peripheral vasodilatation In acute or pernicious beriberi these symptoms appear abruptly with few, if any prodromal signs and the patient may die suddenly of cardiovascular failure Infantile beri beri is usually of this character with an abrupt onset of dyspnea edema, cyanosis cardiac enlargement, and a rapid and irregular pulse Additional symptoms are irritability weakness spasticity, oliguria, and constipation

Peripheral neuritis often accompanies the chronic type of wet beriberi, giving a mixed type, but it also occurs as dry beriberi with few, if any, of the cardiovascular symptoms Many of the subacute *wet* cases lose the edema and the cardiovascular symptoms, leaving a residue of neuritis In dry beriberi the picture is that of a peripheral neuritis, be ginning with the vague and indefinite neuritic manifesta tions already described under the latent or subclinical forms Weakness, tenderness and paresthesias begin first in the feet and legs and develop into a definite neuritis with paralysis and atrophy The Achilles and patellar tendon reflexes, at first exaggerated become diminished and disap pear Burning tingling, superficial hyperesthesia and muscle tenderness are followed by numbness anesthesia, loss of touch discrimination and vibratory sense Atrophy of the muscles may develop until the legs are mere sticks, hidden in mixed cases by the edema, and revealed as the edema disappears Foot drop and contracture deformities may de

velop Ataxia and lack of coordination of a peripheral type dependent on loss of sensory impulses may appear As the process progresses it ascends to involve the upper extremities or even muscles of the trunk and the diaphragm The earlier signs and symptoms may appear in the upper extremities before the process is complete in the legs but the upper extremity is seldom involved before the process is well advanced below Mental confusion may occur in severe cases In many cases the upper arms and hands are involved little, if at all, improvement or recovery occurring naturally (or under treatment) before a more extreme involvement appears In early or milder cases treatment, intentional or otherwise, may cause a prompt loss of symptoms and restoration of function, energetic treatment, a dramatic recovery, often belying the apparently extreme damage In any case however, in which neural degeneration has occurred recovery may be slow and in many severe cases complete recovery may not occur as far as atrophy and weakness are concerned In addition to the peripheral neuritis and cardiovascular symptoms there may be nausea, vomiting, and diarrhea, particularly in the more acute cases Disturbances of vision and hearing, as well as of taste and smell are reported In a few cases, optic neuritis with slight papilledema disappearing on treatment with thiamin, has been observed

The syndromes just described, however, are those of fully developed or severe deficiencies and it is unfortunate that an apparent emphasis is laid upon them by the necessary description of their characteristic and impressive clinical signs and symptoms, an emphasis which is not justified by the relative infrequency of these severe forms Another form of the disease, much more important because of its greater frequency, is the latent or mild deficiency, hypovitaminosis B_1 Unfortunately, emphasis cannot be given to this type by a description of the clinical signs and symptoms They are not impressive and, because they are non specific, to over emphasize them would be to give them greater weight in

diagnosis than they deserve Such emphasis as these milder cases should receive must come from their much greater frequency and the insidious and often unsuspected ill effects they cause in considerable numbers of people It must also be remembered that it is this larger group of mild cases which forms the reservoir from which most of the severe cases develop

Symptoms and signs of mild vitamin B_1 deficiency may be divided roughly into the following groups general or constitutional gastrointestinal neurologic and cardiovascular The general or constitutional include weakness and fatigability loss of weight headache, insomnia, nervousness, irritability and dizziness Recent studies of experimental deficiency in humans suggest that the clinical picture of a psychoneurosis appears in the earlier stages of the deficiency Gastrointestinal symptoms are loss of appetite indigestion, gas and constipation Early neurological symptoms are neuritic pains of various sorts weakness and heaviness of the legs and feet, stiffness cramps in the legs, tenderness of the calf paresthesias such as burning numbness and tingling of the feet and legs The cardiovascular symptoms include palpitation and shortness of breath Slight edema of the legs may be noted

On *examination* in the early stages there is little to be found The subject may show evidence of loss of weight Pressure on the muscles of the legs may cause pain and areas of hyperesthesia or diminished superficial sensitivity to touch and pain may be found Vibratory sense is often diminished The ankle and patellar tendon reflexes may be disturbed Very early they are overactive, later diminished, and finally lost The latter finding however, is usually associated with a definite peripheral neuritis Weakness of the legs may be observed as when the subject attempts to arise from a squatting position

Significant cardiovascular changes are usually lacking unless there is a manifest beriberi of the wet type, generally

acute An exception should be made of these cases in which vitamin B_1 deficiency complicates other forms of heart disease In the milder and earlier cases one may observe an otherwise unexplained tachycardia, a slightly enlarged heart, especially to the right, variable systolic murmurs, alterations in the intensity and character of the sounds, and overactive pulsations There may be a slight elevation of the blood pressure The electrocardiograph is thought by some to reveal changes (previously described) suggestive of B_1 deficiency at an earlier stage than the physical findings

It is obvious that these symptoms and signs are non specific and might be the result of a number of diseases other than B_1 deficiency Nevertheless, when otherwise unexplained and when considered in the light of the dietary history they should lead to a consideration of this diagnosis In particular they are to be given greater consideration when they occur in persons who are prone to develop such a deficiency These include indigents, the aged and infirm, and those confined to institutions who are unable to control their own dietary patients on therapeutic diets, those with organic disease which may affect the appetite, intake, or absorption of food, and persons whose vitamin B_1 requirements are increased as by growth, pregnancy, lactation, fever, increased metabolism, or very heavy work

Special Types Recent investigation has shown that certain types of neuritis, hitherto classified as separate disease are in reality due to B_1 deficiency The two outstanding examples are alcoholic neuritis and the polyneuritis of pregnancy Certain other types of neuritis, such as the polyneuritis associated with some infectious diseases and possibly some of the other disorders associated with chronic alcoholism may also be due to this deficiency Finally, manifestations due to B_1 deficiency may occur with other deficiencies, notably pellagra

Alcoholic Neuritis There now seems little doubt that the polyneuritis occurring in chronic alcoholics, and for a hun

dred and fifty years attributed to the toxic effect of alcohol is in reality due to vitamin B_1 deficiency Clinically and pathologically there is no difference in the neuritis in the two conditions There is adequate explanation for the deficiency of B_1 in the dietary habits of the chronic alcoholic as careful dietary studies of such patients has shown The presence or absence of polyneuritis in any given patient can be explained on the basis of the varying requirements of B_1 depending on the calories consumed as explained in a previous paragraph Here also, careful dietary studies have correlated the presence or absence of neuritis with this relationship between calories and the intake of the vitamin and in this connection it must be remembered that alcohol, while accounting for a considerable number of calories in the diet of an alcoholic, carries with it no vitamin B_1 The gastrointestinal disorders of the chronic alcoholic may act to lessen intake and decrease absorption Alcoholics with polyneuritis allowed to continue their usual intake of alcohol but induced to take a high caloric *high vitamin* diet with supplements of B_1 have shown steady improvement and often cure of the polyneuritis in spite of the continued consumption of liquor In a similar way polyneuritis has been prevented in persons drinking heavily Finally patients with alcoholic neuritis have been cured of the neuritis while taking liquor by administering pure crystalline B_1, a procedure which eliminates the possible effects of other substances contained in supplements such as yeast and yeast products Since in the studies with pure B_1 the possible effects of other substances in the *diet* were not rigidly controlled final proof of the relation of B_1 deficiency to alcoholic neuritis has not been provided Nevertheless the evidence available is sufficiently strong to warrant the clinical assumption that B_1 deficiency is the primary cause of alcoholic polyneuritis It may be added that some at least of the cardiovascular disturbances occurring in chronic alcoholics are the result and manifestation of B_1 deficiency

Delirium Tremens and Korsakoff's Syndrome Korsakoff's syndrome is a chronic delirium usually considered to be associated with chronic alcoholism although it is interesting to note that it was first described by Korsakoff in a discussion of the relation of polyneuritis and psychosis to the vomiting of pregnancy, a syndrome now recognized as being due to B_1 deficiency Polyneuritis often accompanies Korsakoff's syndrome While there is no proof that Korsakoff's syndrome is caused by B_1 deficiency Williams and Spies[4] have observed that patients with this disease who eat a large and well balanced diet supplemented by vitamin products (yeast and liver) recover more often than those who do not

Delirium tremens is an acute alcoholic delirium which occurs in chronic alcoholics, often in association with other complications of chronic alcoholism such as polyneuritis Because of the evident involvement of the nervous system in these cases it might be thought that B_1 deficiency is the cause of the symptoms No substantial body of evidence supports this supposition However, I have successfully treated three cases of delirium tremens (one with optic atrophy, a rare complication) with thiamin, the patients responding dramatically to this alone Others have reported similar effects

Polyneuritis of Pregnancy Polyneuritis has been recognized for many years as an uncommon but serious complication of pregnancy, often with a slow recovery, not infrequently with permanent atrophy and deformity, and at times, a fatal termination Although a dietary deficiency as the cause was suggested many years ago it has until recently been generally attributed to a toxin Recently and on much the same type of evidence as that advanced for alcoholic polyneuritis, it has been quite clearly shown to be due to vitamin B_1 deficiency The disorder is frequently associated with vomiting often the pernicious vomiting of pregnancy It is uncertain whether the vomiting, by interfering with the intake of food, induces the deficiency or whether the de

ficiency which causes anorexia and other gastrointestinal dis orders induces the vomiting Either would account for the chain of circumstances which leads to the development of the neuritis and it is probable that either may initiate a vicious circle which finally leads to a deficiency of thiamin The well known occurrence of minor digestive disturbances and temporary idiosyncrasies of diet, together with an in creased requirement for essential food factors in pregnancy, provides a suitable setting for such a disorder

Neuritis Associated with Other Diseases Peripheral neu ritis has long been known to occur as a complication of other diseases, infections, chronic intestinal disease, meta bolic diseases, especially diabetes other deficiency diseases such as pellagra and sprue, and in the cachexia resulting from malignancies and chronic renal and liver disease It is impossible to state as yet how much of a rôle vitamin B_1 deficiency plays in these disorders and how often yet the effect of these diseases on the intake and absorption of food, and in some cases on the requirements for thiamin makes it probable that in some, at least a deficiency of B_1 is a factor, perhaps the sole factor, in causing neuritis

DIAGNOSIS

Although studies being carried on at the present time on the metabolism of vitamin B_1 and its excretion lead to the hope that a simple and reliable clinical test for its de ficiency will soon be available there is no such test at the present time The diagnosis of thiamin deficiency rests on an analysis of the diet, the symptoms the physical examina tion including the electrocardiogram, certain laboratory tests, and a properly controlled therapeutic test Diagnosis is easily made in the fully developed cases by ordinary physical examination particularly in regions where the dis ease is frequent It is much less easily accomplished in the mild or early cases in the sporadic cases complicating other

disease, and in some of the more atypical forms Because these are of greater importance and more common in this country, greater emphasis will be placed on their diagnosis It is in this group that the laboratory tests though not highly satisfactory are useful

A history of an inadequate diet intake (dietary lack or impaired absorption) may lead to a consideration of B_1 deficiency In the case of the diet we have the advantage of being able to predict with greater certainty a *probable* deficiency *of thiamin (within the symptom range) than is the* case with the other vitamins As the result of his extensive studies on the vitamin B_1 requirements of man as well as animals Cowgill[5] devised a formula for expression of the B_1 needs Williams and Spies[2] and others have prepared similar formulae These formulae can be used to determine the probable adequacy of the diet (in thiamin) taken by a given subject These formulae depend on the fact, discussed under *Nature and Function,* that the requirements of B_1 depend on the metabolic activity, the heat production of the subject, and probably on his weight These formulae have been checked against large numbers of reported diets known to cause, or protect against, beriberi It is apparent, of course, that such requirements as are determined by these formulae are those necessary to protect against beriberi and do not represent optimum requirements or even those sufficient to protect against mild deficiencies Furthermore, the *duration of the abnormal diet must be considered*

The two principal formulae used for calculating the probable adequacy of a diet for B_1 are those of Cowgill[5] and of Williams and Spies[4] The formula of Williams and Spies differs from that of Cowgill in omitting calories derived from fat and neglecting the factor of weight There is also some difference in the values for B_1 assigned to certain foods by these investigators In practice Cowgill's formula is used in the form of a prediction chart* in which the vitamin/

* The prediction chart is given in the appendix

calory ratio is plotted against body weight in kilograms The vitamin intake is determined from a record of the diet consumed If the vitamin/calory ratio of a diet for a given body weight falls well above a predetermined line the diet is considered adequate with respect to vitamin B_1, if below the line, inadequate An in between zone indicates a borderline diet

The formula of Williams and Spies is merely the thiamin/non fat calory ratio, with the vitamin expressed in micrograms In practice the diet consumed over a period of several days is recorded and the calories excluding those from fat, are calculated From the table of vitamin B_1 value of foods the vitamin B_1 in micrograms is calculated and divided by the number of calories If preferred the average daily value for calories and vitamin may be determined and the ratio calculated According to these authors the thiamin non fat calory ratio necessary to protect against beriberi is very close to 0 3 Values slightly less than this indicate a border zone while values below 0 25 may be considered as indicating a deficiency Values in the border zone might be expected to be associated with latent or subclinical cases or hypovitaminosis, without the occurrence of the signs and symptoms of manifest beriberi Williams and Spies believe that their formula is better suited to occidental diets and that their tables of vitamin B_1 values of foods are more nearly correct than some others The use of these formulae and the prediction chart of Cowgill are given in detail in the appendix

The *signs* and *symptoms* of beriberi and mild B_1 deficiency have been given, while the clinical features of multiple peripheral neuritis are well known Little difficulty will be had in recognizing the picture of fully developed beriberi and the various special forms of polyneuritis require only that thiamin deficiency as their cause be remembered In the mild cases however, one must be alert to detect slight symptoms and suggestive physical findings,

regarding them critically because of their non specific nature but giving them due weight as suggesting or indicating a B_1 deficiency—a possibility to be checked by further study

Laboratory Methods The laboratory diagnosis of thiamin deficiency can be made by determining the excretion of the vitamin in the urine, either spontaneously or following the administration of a test dose, and by measuring the amount of pyruvic acid in the blood Other tests have been devised and presented but as yet have not received sufficient trial to be accepted clinically For example, the concentration of thiamin in the white blood cells has been studied and may be a valuable test, as yet it has not become established

The urinary excretion of thiamin can be determined on a 24 hourly basis, or shorter periods can be used and the excretion calculated at an hourly rate Besides the spontaneous excretion the response to a test dose can be measured Because the normal standards and interpretation vary with the method for determining thiamin, they will be summarized following a discussion of the techniques

Thiamin can be determined in the urine by several methods, the most commonly used being the yeast fermentation method of Schultz, Atkin and Frey[8] and the various thiachrome methods * The yeast fermentation method of Schultz Atkin and Frey measures certain fermentation stimulating substances other than thiamin, but the results are expressed in terms of thiamin and have the same relative significance as by the other tests

The normal daily (24 hour) excretion of thiamin by the yeast fermentation method is considered to be above 400 gamma (micrograms) for men and 300 for women Standards for children seem to be essentially the same as for adults By the thiachrome methods an excretion of 60 to 340 gamma (micrograms) constitutes the generally accepted normal range An upper level is of little significance, affected as it is by the intake of more than adequate amounts

* The technique of the test is described more fully in the appendix

The excretion of thiamin is greatly affected by the diet immediately preceding the test a few days of a very low intake giving values below normal but not indicative of a significant deficiency This difficulty, which is often cited as an objection to the use of excretion test, can easily be over come in several ways (1) By a knowledge of the diet If the diet has been poor and the intake low for more than a few days the low excretion indicates a significant deficiency (2) By placing the subject on a normal diet for a day or two before the test This will result in a normal output in subjects whose low intake is temporary and has not caused a significant deficiency It will not affect the low output of a true deficiency subject (3) By the use of a load test

Load tests overcome the influence of variations in intake immediately preceding the test by the administration of a known amount of the vitamin of which the normal sub ject excretes a certain percentage while the greater part is retained by the truly deficient subject Several such load tests have been devised, differing in the amount of thiamin administered, the route, oral or otherwise and the period over which excretion is measured A satisfactory test using the fermentation method for determining the thiamin is that of Pollock,[9] who gives 1 mg intramuscularly and collects the urine for four hours An excretion of 180 gamma or more is normal Najjar and Holt,[10] using a similar method inject 1 0 mg intravenously and find a normal excretion of 120 gamma or more in 4 hours Mason and Williams [11] using the same method for thiamin, give the same dose intramus cularly and find a normal excretion of 20 per cent or more of the test dose in 24 hours

Thiamin deficiency may be detected by measuring the concentration of pyruvic acid in the blood The more severe deficiencies cause an increase in the fasting, basal state, a concentration above 1 2 mg per 100 cc of whole blood con stituting an abnormal level In less severe deficiencies exer cise will disclose the defect in pyruvic acid metabolism

regarding them critically because of their non specific nature but giving them due weight as suggesting or indicating a B_1 deficiency—a possibility to be checked by further study

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Failure of the concentration to return to normal after exercise or an injection of glucose is indicative of a deficiency The increase in concentration of pyruvic acid is not specific for thiamin deficiency Anoxia, exercise, fever, and diabetes are other causes However, due regard for the clinical state of the patient should serve in most cases to distinguish the probability of these other causes and with the proper care in interpretation due all laboratory tests the procedure is of definite value

In addition to the study of the diet and the symptoms and physical signs the therapeutic test is a valuable aid in diagnosis Its greatest drawback is the lack of critical evaluation which so often weakens such a test The availability of the pure vitamin, which should always be employed when treatment is to be used as a diagnostic test, has increased its value and reliability greatly Nevertheless, one must always guard against the dangers of misinterpreting difficulties in absorption, inadequate dosage, and insufficient treatment Other more common errors are the failure to recognize spontaneous improvement, the psychic effect of treatment, particularly when *injections* are given, the effects of other treatment and the presence of other causes for the symptoms causes which may be relieved by nature or by other treatment B_1 deficiency is not the only cause of neuritis, gastrointestinal disturbances, or heart failure, and errors of the kind listed above must be guarded against, particularly in a time of enthusiasm for a new method of treatment

TREATMENT

Treatment is to a high degree specific and consists in the administration of a sufficient amount of the vitamin to relieve the deficiency, restore the body s reserve, relieve the signs and symptoms of the disease as quickly as possible and provide for the maintenance of a continuing adequate intake Accessory factors in treatment are the elimination

of causes for inadequate intake or absorption, the removal, if possible, of conditions causing an increased requirement, the treatment of complications and sequelae, the control of coexisting disease and any symptomatic treatment that is necessary In clearly established cases of the deficiency such treatment is clearly indicated whether the clinical diag nosis is beriberi alcoholic polyneuritis, or some other form Occasions for the treatment of fully developed cases of beriberi and similar types of thiamin deficiency will not occur frequently in this country Treatment will be required mainly for mildly deficient states, for prevention and for the protection of special groups

Preparations Besides pure crystalline thiamin there are hundreds of preparations of the vitamin, many of them con centrates prepared from yeast, wheat germ, liver, rice polishings, etc, containing other substances Some of the preparations are mixtures or combinations of several pure vitamins The preparations vary greatly in potency and in some cases analyses fail to confirm the declared potency Many difficulties arise because of variations and inaccuracies in methods of determining the potency and confusion in the expression of potency (units) Several units are in common use and it is often difficult to convert satisfactorily one unit into another This may be very confusing to physicians who are often unfamiliar with the various units, many of which have been developed in connection with experimental work with animals A further difficulty is that the potency of nat ural substances (yeast for example) varies Not all yeasts are rich in B_1 and the potency may vary with strain, condi tions of growth and similar factors For these reasons phy sicians will do well to use preparations of known potency manufactured by reputable concerns which declare the potency in well established units, preferably in milligrams of thiamin

Pure thiamin, natural or synthetic, when properly pre pared and handled, is of constant composition and potency

Because of this it is to be preferred in many cases but because of the expense, as well as for reasons to be discussed below, may often be replaced by other reliable preparations

Units Because of the existing confusion the following discussion of units is presented Now that B_1 has been identified chemically as thiamin, isolated, and synthesized, the dosage should be expressed in terms of weight (milligrams) To some extent this is being done and some manufacturers are cooperating in giving the dose and potency of their preparations in milligrams or in both milligrams and units However, not all preparations are so described Equally important, many food tables still express the vitamin B_1 content of food in units Consequently, some description of the two commonest units is given *Sherman* units are the older and much of the early work of assaying foods determining requirements and the like was expressed in terms of Sherman units The Sherman unit is based on the growth response of rats placed on graded doses of the substance containing B_1 which is to be tested The unit is the least satisfactory because of variability in the methods and failure to include the influence of other dietary factors now known to affect the result

The standard of weight has now replaced older standards and dosage is ordinarily expressed in milligrams The International unit is now 3 gamma (micro micrograms) of pure crystalline thiamin hydrochloride Earlier it was expressed in terms of a standard substance which was a special absorbate

Conversion of Units It is difficult to convert Sherman units into the older International units or to convert either into milligrams (or gamma) of thiamin hydrochloride because of the variability of methods of determining Sherman and International units Some authorities consider 1 3 Sherman units equivalent to ten older International units,

et cetera In general the following conversion may be con sidered satisfactory for practical clinical purposes

2 Sherman units = 1 old International unit
333 International units = 1 mg of thiamin hydro chloride
1 International unit = 033 mg of thiamin hydro chloride
1 Sherman unit = 0015 mg of thiamin hydrochloride

Or, to put it another way, it takes 600 Sherman units and 300 International units to equal 1 0 mg of thiamin hydro chloride, the probable average minimum daily requirement for an adult On this basis it is seen that it would require 6,000 Sherman units or 3 000 International units to equal 10 mg of thiamin a common therapeutic dose

Most concentrates (yeast, wheat germ, et cetera) are suit able only for oral use They are available in powder and tablets and in capsule form as well, and in many cases are suitable for mixing with foods (milk, et cetera) Certain preparations of liver may be given parenterally but for the latter purpose solutions of thiamin are usually employed Such solutions are available in ampules or may be prepared by dissolving the crystals in sterile salt solution

Prevention Prevention in general is a matter of the proper diet The exact human requirement is unknown but a consideration of the various data indicates that it is approximately 0 35 mg per 1 000 calories (of the diet or energy expenditure) It is affected by weight activity the proportion of carbohydrate in the diet, and perhaps environ mental temperature (sweating) This however is the minimal requirement, and for safety the amount should be increased The recommended allowance of the Food and Nutrition Board calls for 0 5 mg For average men and women this amounts to from 1 2 to 2 3 mg depending on sex and activity For pregnant and lactating women the

allowance is 1 8 to 2 3 mg, while for children amounts from 0 4 mg for those under one year, to 2 3 for boys 16 to 20 are recommended Amounts such as these are perhaps larger than necessary but a good margin of safety is advisable

These amounts can be derived from the daily diet but not with much to spare No foods commonly eaten are very rich in thiamin and it is necessary to depend on more than one or two to get the daily supply In addition, modern occidental diets are weakened in their B_1 value by processes of milling storing, canning and preserving which unfortunately tend to affect particularly certain of the better sources of the vitamin such as the cereals Whole grains are good sources for example, but refined flours and cereals contain almost none Recently, however, much of the bread which is sold has been 'enriched,' bringing the thiamin content to about 1 mg per pound Other enriched cereals are available *A recent governmental order requiring enrichment* of all white bread will provide this good source of thiamin, presumably for the period of the war at least

Foods containing relatively large amounts of B_1 are whole grain or partially milled cereals and products made from them, such as 'whole wheat and 'graham bread,' enriched bread, rolled oats, unpolished rice, old process corn meal, and partially milled rye bread, fresh meat, especially pork and the viscera and eggs, legume vegetables, especially dried peas and beans, and nuts Vegetables in general are from a practical point of view one of the important sources The intake of the vitamin depends not only on the concentration but on the amounts eaten Foods rich in B_1 but consumed in small amounts may be less valuable than food poorer in B_1 content but consumed in large quantities Because vitamin B_1 is very soluble in water the loss in cooking by boiling, a common method with vegetables, may be very large Such foods should be steamed or cooked with little water and when possible the cooking water should

be incorporated in the food as served Prolonged cooking leads to greater loss of B_1 by solution It is relatively insensitive to heat and little loss occurs from exposure to temperatures of 100 degrees during cooking Cooking under pressure however increases this loss rapidly Alkalinity increases the loss and the use of soda in cooking vegetables is very detrimental Preservation by canning (commercial) has little effect on the thiamin content

Carbohydrates increase the requirement of B_1 and diets rich in carbohydrates especially in concentrated forms (sugar, white bread macaroni, potatoes) although valuable for providing energy at low cost and possibly balanced in other directions may result in a B_1 deficiency On the other hand fats have a B_1 sparing action and lessen the need for the vitamin

At the present time several companies are providing flours and breakfast cereals with added thiamin In most cases the amount which is added is no more and often less than that removed from the grain when it was milled Without discussing here the advisability of such processes it may be noted that this process must be kept in mind in calculating B_1 intake in the diet

Protective Treatment This should be given to pregnant and nursing women especially those with gastrointestinal disturbances chronic alcoholics patients with other deficiencies, and those with gastrointestinal disease interfering with the intake and absorption of food those with metabolic and other disorders requiring modified diets patients with chronic infections and febrile illnesses and with cachexias psychiatric patients and psychoneurotics with dietary difficulties and others who for various reasons may be improperly fed

While protection can often be afforded by diet in many instances supplements will be required These can often be

in the form of concentrates of yeast, wheat germ, or simil products However, pure vitamin B_1 may be given and there is reason to doubt the intake or absorption by mout it should be given parenterally In the latter case the dai dose may be provided by large single doses at longer inte vals to avoid the discomfort of injections In the absence urgency, intramuscular or subcutaneous injection will avoi some waste caused by intravenous injection

Breast fed infants are ordinarily protected if the B_1 intak of the mother is adequate and protection should be pro vided by this means except when difficulties of retention o absorption of food exist In these cases parenteral admini tration is necessary and should be given in amounts of o mg daily Bottle fed infants consuming an adequate amoun of milk will receive sufficient B_1 provided the content of th milk is sufficient Factors tending to lessen the latter are im proper feeding of the cow and loss in handling of the milk Infants who, for any reason, are taking inadequate amount of milk or another food or suffer with digestive disturbance should receive supplements of the vitamin, parenterally i necessary As the age increases similar precautions should b observed Children are particularly susceptible to the need for adequate B_1, particularly with reference to norma growth, and require relatively larger amounts of the vitamin than adults It is possible that many of the failures of appe tite and eating in children are the result of mild deficiencie of B_1 which has a powerful effect on appetite

Chronic alcoholics are especially liable to develop B_1 de ficiency While some success may be had by encouraging these patients to partake of an adequate diet the very nature of their illness makes an adequate intake unlikely, while the calories furnished by the alcohol increase the need for B_1 and make the likelihood of deficiency greater Liberal supple ments (10 mg or more daily) are advisable and because of the nature of the illness may require parenteral administra

tion There is some evidence that adequate B_1 intake, by improving general health if for no other reason may make the control of the alcoholism easier In the presence of early signs or symptoms of neuritis, alcoholic delirium, or digestive disturbances energetic treatment with even larger doses is indicated

In patients with other deficiency diseases, especially pellagra adequate provision of B_1 should be made, because in these cases the diet is usually lacking in several necessary substances It is particularly needed when nicotinic acid or similarly highly purified products are used Everyone with experience in deficiency diseases has seen the symptoms and signs of B_1 deficiency appear in pellagrous patients treated and improving with nicotinic acid

Patients with gastrointestinal disease require special protection because B_1 intake is apt to be lessened by anorexia, loss by vomiting and diarrhea, poor absorption, therapeutic diets poor in B_1 Such patients may well be given amounts considerably larger than the usual protective dose up to 5 to 10 mg daily Because such patients are liable to be deficient in other respects as well, concentrates of the entire B group are used to advantage and when pure B_1 is used caution must be exercised to supply the others as well Difficulty with absorption and vomiting necessitate parenteral administration

In diabetes and similar metabolic diseases it is usually possible to adjust the dietary so that adequate B_1 is provided When control of the primary disease makes it necessary to employ rigid diets deficient in B_1 supplements should be provided and the occurrence of signs and symptoms of so called diabetic neuritis or cardiovascular complications should always lead to a careful check of the diet with respect to its B_1 content Consider the *quantitative* aspects of the diet in particular in the case of all therapeutic diets

Acute infectious diseases of short duration require little

in the way of protection *provided an adequate store of the vitamin existed before the onset* However, proper intake secured by diet or supplements may avoid the loss of appe tite and the development of a deficiency Infections of long duration, such as tuberculosis, may have resulted in a de ficiency and supplements may be required to restore the body reserves

Finally, many patients with the cachexia of chronic ill ness such as cancers, chronic nephritis, heart disease, mental disease, and senility should be assured of adequate intake of B_1, by diet if possible, if not, by supplements

Curative Treatment In clear cut cases of thiamin de ficiency in the *adult* 10 to 20 mg of crystalline B_1 (thiamin) should be given daily as soon as the diagnosis is made In some cases such as severe neuritis and cardiac failure (beri beri heart), *somewhat larger doses (20 to 40 mg) may be* given It is doubtful whether any more than this is worth while and in most cases larger amounts are wasteful Excep tionally, as a matter of trial in cases which seem to offer difficulties in utilization or in conditions whose relation to B_1 deficiency is doubtful but worthy of investigation, much larger doses may be given

The drug may be given by mouth or parenterally In the milder cases and when there is no difficulty in oral adminis tration or absorption it may be given by mouth The use of the larger tablets (3 mg or more) is advantageous Paren teral administration should always be employed in severe cases of neuritis, cardiac failure, or those with nausea, vomit ing and diarrhea Also, this method is to be employed when the patient is very ill, when ingestion may fail because of lack of cooperation or carelessness, or when there is inter ference with absorption It may be given intramuscularly or intravenously Ampules containing sterile solutions are available in different sizes or the crystals may be dissolved

Parenteral injection ensures prompt action adequate dosage and certain absorption In many cases it will be sufficient to give only the first few doses by injection after which sufficient improvement will have occurred to allow the drug to be continued by mouth

After the major symptoms have disappeared the crystalline B_1 may be supplemented by various concentrates and substances rich in B_1 These include dried brewers yeast, powder or tablet wheat germ extract of rice polishings and similar preparations Dried brewers yeast and wheat germ are more commonly used in this country Dosage should be based on the thiamin content and should be equivalent to not less than 10 mg of the latter daily Since the content of B_1 in these preparations is as yet ordinarily given in various units (International, Sherman et cetera) it is necessary to convert these into mg (See note on conversion of units on page 64) In general terms about 6 ounces of a good quality of brewers yeast or of wheat germ should be given daily

Besides the vitamin however given the patient should be placed as soon as possible on an adequate well rounded, high caloric diet This is of great importance because it is desired not only to cure the existing disease but to establish a diet which will protect the patient against the return of the disease and other nutritional disorders Furthermore although B_1 will correct the disorders and cure the symptoms resulting from its lack most patients with B_1 deficiency suffer from other nutritional disorders as well, disorders which B_1 will not relieve, and which will interfere with recovery from that deficiency and prevent the complete recovery of the patient The use of the various concentrates which contain other vitamins and essential substances as well as B_1 are valuable for the same reason *

* This statement should not be construed as a recommendation for a large group of preparations consisting of mixtures of vitamins often with minerals which are offered by drug companies Besides other objec

A diet containing 4000 5000 calories, with liberal amounts of fresh vegetables and fruit (much of it raw), *fresh meats*, especially liver and pork, whole grain cereals, legume vegetables, milk and eggs, is advisable In many cases such a diet will be taken readily, even avidly, after an improvement of symptoms has resulted from treatment with the vitamin In some cases, however, the effect of accompanying diseases, gastrointestinal lesions, et cetera, will interfere with an adequate diet and it will be necessary to continue supplements over a long period Especial care must be taken in chronic cases that adequate treatment is continued over a time sufficiently long to secure a cure or the maximum improvement possible This is particularly true in the cases of severe neuritis, which may respond slowly and show complete recovery only after months, and in patients with gastrointestinal lesions in whom absorption is poor In the latter absorption may be so inadequate that they require special dietary attention and adequate supplements over indefinite periods Finally, in the more severe and acute cases the prompt and gratifying response to early treatment should not cause one to neglect continued treatment, especially by means of diet which is necessary to complete the cure

Infants Older children are treated as adults but infants in particular require prompt and energetic treatment if they present the manifest symptoms which constitute beriberi In these cases parenteral treatment is advisable The requirements of infants are relatively greater than for adults and 5 to 10 mg should be given intravenously or intramuscularly twice daily When this cannot be done, or in the milder cases crystalline vitamin B_1 can be added to the formula and in still milder cases after the initial injections

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tions these while containing several vitamins usually have any individual one in such small quantities that adequate dosage is impossible except at excessive cost and trouble

concentrates may be given by mouth, alone or mixed with the food In the Far East tiki tiki, an extract of rice polishings, is used successfully As in adults the diet should be altered to provide a liberal intake in the food In nursing infants energetic treatment of the mother, upon whose deficiency is contingent that of the child will help the child However in the more severe cases treatment of the mother should not be solely relied on and the infant should be treated directly

Other Treatment Although vitamin B_1 in some form or other is the only specific treatment for the deficiency, other forms of treatment will often prove helpful in relieving the patient, preventing complications and shortening the period of disability All patients with the severe forms of the deficiency should be at rest in bed to lessen the likelihood of cardiovascular collapse, promote the relief of symptoms, and hasten recovery Rest diminishes the need for the vitamin and hence indirectly increases the effectiveness of the dose It also serves to protect weakened or paralysed muscles In acute heart failure venesection may be an immediate life saving measure Digitalization is usually not effective and its action is no faster than that of thiamin itself which is to be preferred Diuretics in particular the newer mercurial diuretics, may be used to relieve edema but the effect of adequate doses of thiamin is nearly as rapid Large effusions may often be relieved most rapidly by tapping but this results in a considerable loss of protein from the body which may be undesirable in these patients

Patients with neuritis often obtain considerable relief from pain through cradles though the more severe pain is usually promptly relieved by adequate doses of thiamin provided proper absorption is secured In the latter stages of cure of severe neuritis in which there may be a complete paralysis, massage and careful and progressive exercise is

beneficial Splints, casts, and similar orthopedic methods may be required and tendon lengthening may be needed

Analgesics may be required with severe neuritis but sel dom are needed for long In acute, severe stages weaker drugs than codeine or morphine are of little use, being no more effective than thiamin itself Codeine and morphine may be indicated temporarily but should be discontinued as soon as possible, because of the tendency to habituation

Little need be said here of the treatment of concurrent disease The important thing is to emphasize that certain diseases predispose to the development of B_1 deficiency by increasing the needs for the vitamin, lessening its intake or interfering with the absorption To these should be added a special group of those diseases which of themselves require special diets which unless care is taken, are apt to be inade quate In any of these diseases the longer they are present the greater likelihood there is that a deficiency will result In chronic disease particularly the need for prompt and en ergetic treatment and alertness to provide adequate nutrition should be emphasized Diseases which increase the demand are those which speed up metabolism such as thyrotoxicosis and fevers Those leading to lessened intake and often de ficient absorption are gastrointestinal disorders, food sensiti zation, cachexias of malignancy, heart disease Those requir ing special diets which may include some already mentioned are diabetes peptic ulcer, obesity, and heart disease In all these divisions the possible need for the parenteral adminis tration of B_1 must be recognized

Prognosis Recovery from vitamin B_1 deficiency, even in severe cases is often prompt, even dramatic when proper treatment is instituted Even in cases of severe heart failure or acute severe neuritis, improvement may occur in a few hours and the symptoms disappear in a day or two The same is not true in chronic cases, however Although partial relief, especially from pain, may occur rapidly, progress thereafter

is slower and for weeks or months the patient may complain of annoying paresthesias and weakness in the extremities As already indicated in some cases of paralysis, permanent residual weakness may result The results in cases of neuritis are, of course, dependent on the degree of damage to nerve fibers and cells Those severely damaged recover slowly, the time needed being related to the speed of regeneration from the nerve cell toward the periphery, which is slow This difference between acute and chronic deficiency is the basis for certain doubts of the specificity of the nerve changes Gastrointestinal symptoms are ordinarily relieved quickly and completely the anorexia due to the deficiency often responding in a few hours

Contra indications and Toxic Effects As far as is known, there are no contra indications to the therapeutic use of vitamin B_1 even in pure form and no toxic effects in single doses or from cumulative effects Experimentally animals can be poisoned and even killed by thiamin (vitamin B_1) but the amounts needed far exceed any conceivable thera peutic doses Amounts as large as 0 5 Gm have been given daily for months without any apparent toxic effect and intravenous injections of even 20 times the usual dose have caused no untoward reaction

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4

Nicotinic-Acid (Niacin) Deficiency

Pellagra—Pellagra sine Pellagra

HISTORY

PELLAGRA has been considered a deficiency disease since the early part of this century but particularly since the work of Goldberger Although other theories postulating infectious and toxic causes have been advanced in the past and are still advanced, it is now generally accepted on almost conclusive evidence that it is a deficiency disease

In the confusion which has existed in respect to the B Complex of vitamins and still exists to a considerable extent clinically it may be premature to consider pellagra the result of the deficiency of a single member of this complex of vitamins Nevertheless the discovery that nicotinic acid is the primary, if not the sole, factor concerned in the prevention of black tongue in dogs and is responsible for the principal symptoms of pellagra in man is sufficient justification for the present for considering pellagra the clinical expression of nicotinic acid deficiency

Other members of the B complex may be and undoubtedly are often deficient in patients with pellagra The deficiencies may even contribute to the symptoms Thiamin may be deficient and produce symptoms yet these are not symptoms of pellagra but symptoms of B_1 deficiency complicating pellagra In the same way the possible symptoms produced by a deficiency of other members of the B group may complicate pellagra as has recently been shown to be

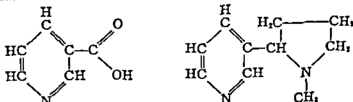
the case with riboflavin Therefore, I prefer for the present to consider pellagra the expression of nicotinic acid deficiency, recognizing that it may be and often is complicated by other deficiencies So far conclusive evidence of a necessary relation to the human organism of members of the B complex has been shown for only B_1 (thiamin), riboflavin, and nicotinic acid

Pellagra has undoubtedly existed for many years, appearing with other deficiencies produced by war, famine, poverty, and disease Its clear recognition as a separate disease, however, dates from the description by Casal in 1735 who labelled it *mal de le rosa* It was well known in Italy and it was an Italian, Fripolli, who named it pellagra (rough skin) Although apparently not recognized in this country except for isolated cases until 1906 it undoubtedly existed earlier and was known to physicians who were unaware of the description by European physicians and the names given to it by them Even casual reading of case reports or text books of an earlier day, or the description by physicians or laymen of disease among troops and in prisons during the war between the States will allow one to recognize cases of the disease In more recent years many reports of epidemics as well as endemics and sporadic cases have established it as one of the most common nutritional diseases, at least in manifest form, in this country

NATURE AND FUNCTION

Nicotinic acid is an organic compound related chemically to nicotine but with very different physiologic properties *

* Nicotinic acid is 3 pyridine carboxylic acid and has the following structural formula



It has long been known having been discovered and pre pared by Hyber in 1867, and isolated from yeast concen trates in 1913 by Funk who thought it might be the anti neuritic factor (B_1) Funk failed to discover its relation to pellagra Little more was done with it until Warburg and Christian found it to be a constituent of one of the co enzymes Subsequent progress was rapid and demonstrations of its curative action in black tongue of dogs by Elvehjem was rapidly followed by demonstration of its effectiveness in pellagra Nicotinic acid and nicotinic acid amide are available in pure form for use clinically

Nicotinic acid in the form of the amide is combined by the body with phosphoric acid, an amino acid, adenine, and a sugar ribose to form a coenzyme * It thus is a part of one of the essential enzyme systems, used for transferring oxygen in oxidations concerned with fundamental life processes in the cells Lack of nicotinic acid prevents the formation of the coenzyme because the body cannot synthesize nicotinic acid However despite the fact that much is known of the man ner in which this coenzyme acts in the biologic processes of cells and isolated tissues almost nothing is known of the relation of this action, or disturbance in this action, to the pathologic physiology and structural changes which char acterize the disease, pellagra Just how the inflammation of epithelial tissues (dermatitis, glossitis proctitis) and the changes in the nervous system (dementia) are to be related to a disturbance in this enzyme system remains to be deter

The relation of this compound to nicotine is seen from the formula of nicotine which is given here for comparison So far as is known nicotine is not utilized by the body in any way related to nicotinic acid Certain related compounds such as ethyl nicotinate nicotinic acid N methyl amide nico tinuric acid etc possess certain activity others such as pyridine picolinic acid isonicotinic acid trigonelline etc do not Apparently whether or not such compounds are active depends on whether the body can convert them to nicotinic acid

* Nicotinic acid is used to form two coenzymes known variously as coenzyme I Harden and Young s coenzyme cozymase coenzyme II or Warburg s coferment They are better designated by the chemical names diphosphopyridine nucleotide and triphosphopyridine nucleotide re

mined The fact that the enzyme system in which it is incorporated includes other essential substances, available to the body from external sources only suggests that a deficiency in these other substances may disturb the system and be responsible for some of the disease manifestations commonly associated with a deficiency of nicotinic acid Those due to lack of nicotinic acid are, however, the principal ones and for the present it seems best to consider other deficiencies if present as conflicting deficiencies

Nicotinic acid and nicotinic acid amide are soluble in water and easily and quickly absorbed from the gastrointestinal tract Most of the nicotinic acid appears to be present in the body as the amide to which the acid seems to be converted soon after absorption Furthermore, it is probable that the greatest part is present in the cells as the coenzyme, relatively little remaining as the amide or free acid Judging from animal studies relatively large amounts are found in the *liver*, *muscles*, and *kidneys* Storage appears to be better than is the case with thiamin or vitamin C The amount in the blood is relatively small (in man) and nearly all is present in the cells in the form of the coenzymes Small amounts of the amide and perhaps the free acid are found in the plasma, possibly as a 'transport portion Feeding large amounts of nicotinic acid or the amide increases the content of whole blood and cells even in normal persons but not beyond a certain maximum When this is reached continued administration fails to increase the concentration and the excess is probably excreted unchanged It is probable, though not certain, that a similar process takes place in the other tissues During a deficiency the blood holds on to its nicotinic acid (in the form of coenzyme) so tenaciously that with even manifest pellagra the concentration in the whole blood often fails to fall below that observed in normal subjects Hence determination of the concentration in the blood is of no value in diagnosis

Nicotinic acid appears in the urine as the free acid, as

the amide, and also, under certain circumstances at least, as nicotinuric acid and trigonelline It is these latter substances which make the determination of nicotinic acid in the urine of doubtful value in diagnosis, at least for the present Although nicotinuric acid and trigonelline can be converted to nicotinic acid and measured as such, both are excreted following the ingestion of such substances as tea and coffee, nicotine, cocoa, and perhaps other substances in the diet It is difficult, therefore, to determine the significance of total nicotinic acid excretion

In contrast to most other vitamins nicotinic acid has a certain pharmacologic and, in a sense, mildly toxic action That is to say, it causes certain physiologic reactions in individuals who are not deficient in it These occur whenever a single dose exceeds a subject s tolerance or when smaller doses, repeated at frequent intervals exceeds the tolerance within a given time, probably by increasing the blood concentration beyond a given level Tolerance varies but for most persons is in the neighborhood of 50 mg in a single oral dose, 10 mg intravenously The symptoms consist of flushing, burning and tingling of the skin, beginning in a typical case in the head and neck and passing progressively down the trunk and extremities With this there is a diffuse redness of the skin, resembling even a scarlatiniform rash There is obviously a dilatation of the superficial vessels but no change in blood pressure, temperature (body) or respiration The effect passes off in a few minutes Following large intakes over some days the effect seems to be produced by smaller doses Recently Najjar and Holt have reported two fluorescent substances, named by them F_1 and F_2, which appear to have some relation to nicotinic acid F_2, developed only on addition of alkali is present normally in the urine but lessens and disappears in nicotinic acid deficiency, while F_1 normally present in small amounts or not at all, increases in the deficiency Their exact significance is yet unknown

Because the amide does not cause these symptoms it is probable that the effect is due to an inability of the body to change the free acid to amide with sufficient rapidity This is supported by the fact that glycine administered *with the nicotinic acid will* prevent the symptoms

No serious toxicity has been observed and it is unlikely that any will occur except with relatively enormous doses

Nicotinic acid in the form of the coenzyme (known as factor V) is necessary for the growth of certain micro organisms, a fact which illustrates its fundamental importance in life processes This function is also the basis for certain biologic tests for nicotinic acid It is of some interest that the bacteriostatic action of certain chemotherapeutic drugs against certain micro organisms is due to the action of the drug in blocking the nicotinic acid (as coenzyme) for use by the bacteria The exact significance of this reaction in the human organism in connection with the use of chemotherapeutic agents is not yet clear

PATHOLOGY AND PATHOGENESIS

The individual pathologic findings in pellagra are in no way specific but nevertheless their composite appearance is usually sufficiently characteristic for the diagnosis to be made in a person dying of pellagra According to Eddy and Dalldorf the early lesions in the skin and colon present a highly characteristic if not specific appearance Grossly the characteristic changes are inflammatory lesions of the skin, inflammation of the mucous membrane surfaces of the gastrointestinal tract from mouth to rectum, and a vaginitis in women In chronic cases the lesions of the skin may change, the inflammatory process subsiding, leaving either a roughened thickened skin or a thin, atrophied one, with increased pigmentation in either case Lesions in *the colon show similar changes in the later stages*

The microscopic lesions in the skin and colon said to

be characteristic in the early stages were first described by Denton and have been confirmed by Eddy and Dalldorf, whose description is the basis of this account In the skin they precede the erythema and consist of edema of the papillae, dilatation of the papillary blood vessels and de terioration of the superficial fine collagen layer of the corium Later, the capillary endothelium is swollen and the finer collagen fragmented Vesicles are found and be come infected causing sloughing of the epidermis Still later the superficial layer becomes atrophic or has an overlying thickened horny layer There is an increase in pigment the nature of which is uncertain In the oldest lesions there is atrophy of the rete malpighii, and a thin epidermis

The colon walls are thickened, inflamed with patches of pseudo membrane and a stippled appearance due to small gray bodies seen on histologic examination to be cystic crypts of Lieberkuhn said by Herzenberg to be practically pathog nomonic of pellagra occurring in only one other disease, sprue In the late stages there is atrophy of the mucosa

It is difficult to see how these lesions, those in the colon at least can be considered early lesions Presumably the material was obtained at necropsy from subjects who had died of pellagra In any event by the modern point of view they are expressions of a severe and advanced deficiency

Nervous system lesions consist of scattered degeneration of axis cylinders of the pyramidal cells of the cortex and a myelin degeneration of fibers in the spinal column mostly in the posterior columns, the lesions resembling those of subacute combined sclerosis Peripheral lesions are uncom mon None of the nervous system lesions is specific

INCIDENCE AND EPIDEMIOLOGY

Pellagra is found in endemic or epidemic form in a good many parts of the world and by its nature may occur spo

radically anywhere It is said to be frequent in Egypt, the Balkans, Spain, Italy, and the Soviet Union It is a common disease in the United States and although undoubtedly more frequent in certain regions such as in some of the Southern States, it is by no means confined to those regions The in cidence is thought by some to be decreasing in this coun try and personal observation suggests that this is true if the incidence of fully developed cases is meant However, re porting of the disease has been notoriously poor and incom plete even in those states where reporting is required and if one includes subclinical stages of the disease, or nicotinic acid deficiency, as should be done, there is no way of know ing how frequent it is No doubt cases must be present in considerable number in all parts of the United States

Pellagra is primarily a disease of the poor although spo radic cases can occur as the result of a wide variety of 'con ditioning factors,' such as carcinoma of the stomach, alco holism, food faddism, etc Also among certain groups dietary *habits* and ignorance rather than actual poverty may main tain a reservoir of mild endemic deficiency from which develop a certain number of fully developed cases as indi vidual circumstances give occasion The disease is most fre quent in the middle years and women are more often af fected than men It is rather uncommon in children Negroes and whites are both affected and there is probably little or no true racial variation The greatest incidence in this country is in the spring and early summer which is prob ably the result of the lag in the development of symptoms from deficits occurring during the winter months Sunlight at this season probably hastens the appearance of certain symptoms (dermatitis) but does not cause the disease

SYMPTOMS AND SIGNS

The signs and symptoms of fully developed pellagra are familiar to most physicians though they may occasionally

be missed in regions where endemic pellagra does not occur and only occasional sporadic cases are seen Chronic and mild or latent cases are much less easily recognized though they are more important because of their greater frequency

Typical severe, acute pellagra is characterized by a dermatitis a glossitis and stomatitis, diarrhea and mental symptoms, often delirium Not all the symptoms need be equally severe, the dermatitis and gastrointestinal symptoms being the most frequent There may be fever and extreme prostration ending with death Although such cases may appear to develop suddenly they are preceded in nearly all cases by a prodromal period during which the patient complains of weakness, lassitude anorexia, indigestion, mental depression or irritability and a tendency to diarrhea Most of the cases in endemic areas appear in the spring and in many cases are the final expression of a disease appearing each year for several years in gradually more severe form until the acute attack develops Sporadic cases often do not have this seasonal incidence

In addition to these severe acute cases many instances occur in which the disease, while presenting typical and easily recognizable signs is much more mild In them there is an acute, typical dermatitis, moderate glossitis, and diarrhea, anorexia and indigestion, weakness, and nervousness or mental depression without the severe prostration, fever, delirium and grave prognosis of the severe cases Such cases not infrequently clear up, or partially clear up spontaneously, only to recur, perhaps in a more severe form the following year or settle into a chronic stage Little difference is found between children and adults, men and women

Digestive System Glossitis and diarrhea are the outstanding symptoms of this system The glossitis and stomatitis, vary from a mild redness soreness and smoothness of the tongue and mouth to an extreme inflammation with fiery redness of the mucosa, atrophy of the papilla of the tongue, dryness ulceration and secondary infection of the tongue

and buccal mucosa The sore tongue is one of the first signs of the disease The pharynx and esophagus are involved as well as the mouth and the taking of food and swallowing may be difficult or impossible With the stomatitis there may be salivation Nausea and vomiting are seen in the more severe cases The diarrhea ranges from several loose stools a day to 15, 20, or more watery, bloody passages with mucus and tenesmus There is often a severe proctitis with red ness, burning, and itching about the rectum In women there may be a vaginitis as well

Skin The dermatitis is perhaps the most characteristic symptom though it may be absent entirely The acute le sion in the early stages is a bright red erythema resembling sunburn, occurring characteristically over the exposed parts of the body with a bilateral symmetry which is quite accu rate The commonest sites are the back of the fingers and hands, the forearms, the dorsum of the feet and ankles and the ' V ' of the neck In the latter area it forms the so called "necklace or Casal s cravat There also occurs a butter fly' lesion over the bridge of the nose and cheek, but this is perhaps due to associated riboflavin deficiency In the beginning the skin is red and slightly swollen, it itches, burns and stings In this stage the effect very closely re sembles sunburn The margins of the areas are sharply out lined and slightly raised but as a rule the periphery is less severely affected than the center The lesion may progress to the formation of vesicles and bullae with cracking of the skin, exudation of a sero sanguineous exudate, and crust ing Secondary infection and ulceration are nearly always present to some degree in these cases With improvement the skin becomes dry, less red, and the surface desquamates leaving a pinkish thickened skin with a pigmented edge Following repeated attacks and in chronic cases the pig mentation is more general and the skin, especially over such parts as the elbows, knuckles, and knees is brown, dry, scaly, and thickened In recently healed cases the skin

festations of the disease is not uncommon in regions of endemic pellagra Such cases are to be distinguished from the subclinical or latent cases which scarcely emerge above the clinical horizon and fall between the former and the acute cases These chronic pellagrins *may have chronic* dermatitis, may suffer with mild forms of diarrhea, or stomatitis, or may exhibit mild and indefinite mental symptoms The dermatitis is frequently present as a roughening and thickening of the skin with dryness and scaling over the backs of the hands, dorsum of feet and ankles, and over the knees and elbows In the exposed areas there is frequently some brownish pigmentation Erythema is slight or absent and vesiculation, ulceration, and crusting are lacking In these cases the bilaterally symmetrical distribution and the well defined border are less apparent though the hint is usually there The diarrhea seldom exceeds 3 to 5 stools a day, the stools soft rather than liquid There is usually associated indigestion and often anorexia which may be due to associated deficiencies The glossitis is slight or mild, often with little atrophy and but slight redness and soreness, which is confined to the tip and sides With the glossitis there may be a low grade proctitis and vaginitis Mental symptoms comprise irritability, change in disposition, faulty memory, poor powers of concentration, and sometimes *more significant changes such as depressions or* even mild delusions—symptoms simulating those of a psychoneurosis and whose essential origin in a vitamin deficiency is shown in the favorable response to treatment, forming a large part of so called cases of pellagra sine pellagra

Latent or Subclinical Pellagra (Nicotinic Acid Deficiency) As is the case with other deficiency diseases, early or mild, subclinical or latent pellagra must occur under conditions which cause a deficiency of nicotinic acid not severe enough, or of too short duration, *to produce the clinical picture of* the disease There is reason to believe that such cases far *outnumber* those of clinical pellagra but, in the absence

of suitable laboratory tests to demonstrate the deficiency, their detection is difficult and knowledge of their exact number difficult to estimate For the present their recognition depends on the detection of the earliest and mildest of the clinical symptoms and signs, an analysis of the diet and the results of a therapeutic test as will be discussed under diagnosis Such cases are to be distinguished from the chronic cases which present definite, if mild or atypical, manifestations and in most instances a history of previous acute or manifest attacks

Special Types Special types of pellagra are described These are cases of pellagra in which the deficiency instead of being of simple dietary origin is conditioned by some other factor, usually a disease, such as 'alcoholic pellagra but they differ in no essential respect from endemic pellagra Such deficiencies as occur are due to the effects of the associated disease Because these forms occur as sporadic cases, often in areas in which endemic pellagra is not found the diagnosis is less easily made by those unfamiliar with the disease

DIAGNOSIS

The diagnosis of pellagra rests as it does in other deficiency diseases on a knowledge of the diet the detection *of signs and symptoms of the disease whether they be* slight or unmistakable, the results of laboratory tests and the response to a therapeutic test In pellagra the laboratory tests are as yet not well established

Dietary Inadequacy Dietary inadequacy can be discovered from a careful scrutiny of the diet in the case of pellagra but not with the same degree of certainty as in the case of vitamin B_1 Recently the content of nicotinic acid has been determined for many foods in terms of absolute value (milligrams) so that it is possible to measure quite accurately

the nicotinic acid content of the diet However, it is not known certainly whether other substances are concerned in the production of pellagra, and if they are, what their number and relationship may be Nevertheless, a careful analysis of the diet will help in the diagnosis of a possible deficiency, and a small intake of such foods as fresh meats, milk, eggs, and green vegetables will be suggestive Not all vegetables are a good source of nicotinic acid, but some are and failure to include a fair amount and variety is an index of possible shortage unless the diet has much meat and dairy products

In pellagra the occurrence of complicating diseases or pathologic states is of particular importance because of the frequency with which these predispose to pellagra and the frequency with which sporadic pellagra occurs in association with other disease

Symptoms and Signs of Pellagra

Dermatitis Most of the symptoms and physical signs have been described In acute, fully developed cases, the characteristic, symmetrical dermatitis, diarrhea, and mental symptoms can scarcely be missed The significance of diarrhea and psychic disorders should not be missed in patients without dermatitis Dermatitis is often absent or slight in patients little exposed to sunlight While the dermatitis is commonly confined to exposed areas it may involve areas covered by clothing Conversely it may be lacking in some parts which are exposed In early or mild cases the inexperienced examiner may miss a slight erythema and its symmetrical distribution With a distinct dermatitis the border marked by swelling is usually sharply defined but in these cases there is often a bordering zone of slight erythema which merges imperceptibly into the adjacent region Sunburn is too often the mistaken interpretation of the inexperienced, hasty or careless examiner In severe cases with vesiculation, ulceration and secondary infection the cause of the dermatitis may be missed if attention is focused on the process

as a "skin disease and the general examination, which would reveal the glossitis, mental signs, et cetera, is neg lected Failure to examine the vagina, rectum, perianal region and scrotum may cause one to miss inflammatory changes in those areas Such lesions may be present without a dermatitis of the extremities In chronic cases the rough ness, pigmentation, thickening of the skin in sites of pre dilection, often with the telltale shadow of their symmet rical distribution, may be very helpful and suggestive

Glossitis and Stomatitis The glossitis of pellagra differs from that seen in certain other nutritional states by the greater inflammatory reaction In the earliest stage a slight redness just at the tip, then the sides is apparent With this and even preceding it there may be indentations from the teeth which some think represent one of the earliest changes Atrophy of the papillae occurs but is less constant and less severe than in other types of glossitis Although glossitis may be absent in the presence of other signs of the disease and may vary in intensity it is most often pres ent to some degree and is one of the most constant and earliest signs Recently Kruse has described in great detail not only the gross changes but those changes in the papillae observable with the microscope under slit lamp illumination Acute and chronic forms, each of varying intensity or severity, are described and acute forms may be superimposed on the chronic Different areas may show different stages In the acute forms hyperemia and proliferation, hyper trophy and extinction of the papillae occur successively in the chronic forms hyperemia and proliferation infiltration and atrophy The fungiform papillae are affected first Restoration occurs with specific treatment but may require very long periods (months) in chronic cases Whether these changes especially those observable microscopically are sufficiently specific for diagnostic reliability is not yet certain

Nervous Manifestations In acute cases a toxic delirium characterizes the mental changes in the more chronic types

of apathy, loss of memory, depression, and disorientation Many of the mild and especially the latent cases present the symptom complex commonly referred to as a psychoneurosis As previously indicated, symptoms referable to the peripheral nervous system are probably due to associated deficiency (B_1) However, the signs of combined system cord disease, and disturbances of sphincter control seem to be a part of the pellagra itself

A diagnosis of nicotinic acid deficiency must be made from an analysis of the diet, the symptoms and physical signs, and a therapeutic trial At the present time there are no laboratory tests for the diagnosis of pellagra Such tests, while little needed for the well developed cases would be very useful in detecting mild or latent cases Tests for porphyrinuria have proved unreliable, and the determina tion of the nicotinic acid concentration in the blood by chemical or biologic tests fails to show consistent decreases even in patients with pellagra So far the measure of the excretion of nicotinic acid in the urine with or without a test dose has not proved practical

Laboratory and Special Tests

Several tests using the urinary excretion of nicotinic acid have been reported, that by Perlzweig and his associates being the more completely designed for clinical use The excretion of nicotinic acid and derivatives (with trigonelline determined separately) is measured by the author s tech nique following a test dose of 500 mg of nicotinic acid amide However, the procedure is rather involved, requiring a special three day diet and the collection of two 12 hour urine samples In patients with difficulties in intestinal absorption intravenous administration is recommended There is a rather wide range of excretion in individual sub jects and as yet no well established standards have been set

Najjar, Stern and Holt have utilized the variations in the presence and amounts of the fluorescent substances F_1

and F_2' described previously as a test of nicotinic acid deficiency In nicotinic acid deficiency (pellagra), F_2, which is abundant in the urine normally, lessens or disappears, and F_1 which normally is absent or present only in small amounts, increases greatly These substances are detected and measured with a fluorimeter, as yet the test s exact clinical value and generally accepted standards have not been set up

TREATMENT

The introduction of nicotinic acid has revolutionized the treatment of pellagra In the vast majority of cases it will promptly, even dramatically relieve the acute symptoms, bringing about an early clinical cure in the milder cases and a progressive, if somewhat slower, return to normal in the more severe ones Dependence should not be placed on it alone, however As in the cases of other deficiency diseases proper treatment requires the institution of an adequate and complete diet in addition to the administration of the purified specific substance Such dietary treatment and correction should be instituted as soon as possible, the pure or concentrated preparations being used to treat and to relieve the effects of the deficiency and restore the body stores to normal the cure being completed and maintained by a proper dietary intake Only when there is difficulty in absorption or utilization or in the presence *of excessive demands for the vitamin (apparently uncommon* in the case of nicotinic acid) should it be necessary to continue supplements of the vitamin in pure or concentrated form In the course of treatment the use of a concentrate or product especially rich in nicotinic acid but also containing other necessary accessory substances which may be involved in the disease is helpful The reasons for this procedure have been given elsewhere but are particularly applicable in the case of pellagra because of the frequent association with this disease of other deficiencies

and even more because of uncertainty regarding the exact rôle (mechanism) of nicotinic acid in the prevention and pathogenesis of this disease Indeed, as already mentioned, nicotinic acid alone, in pellagra patients continuing on a pellagra producing diet, may fail, even with increasing doses to prevent a recurrence of the disease

Nicotinic acid and nicotinic acid amide are available in pure form In general the amide is to be preferred because it is just as effective as the acid and is free from unpleasant reaction Besides the free acid and the amide a number of related substances including coramine (nicotinic acid N diethyl amide) can be used but offer no particular advantage and are less effective Dosage is expressed in milligrams and there is fortunately no confusion with other units *

Nicotinic acid and nicotinic acid amide can be given by mouth, in capsules, or in solution In solution it can also be given subcutaneously, intramuscularly, or intravenously When given parenterally the amount which will give un pleasant reactions is much smaller than when given by mouth Solutions of the acid and the amide are quite stable but fresh solutions or material from ampules should be used for parenteral injection

Nicotinic acid and the amide are also supplied in the form of concentrates such as yeast Such products are valu able sources and are to be preferred under many circum stances as will be shown below However, they, of course, contain other vitamins

Prevention For the prevention of pellagra and nicotinic acid deficiency in otherwise normal individuals nothing more is needed than an ordinary, good diet, adequate in calories and containing usual amounts of a variety of fresh or properly canned vegetables, fresh or canned meat at least several times a week, milk, and eggs Conversely the diet should not consist mainly of highly milled cereals,

* Except for the short period during which riboflavin was believed to be the antipellagra (or vitamin G) factor and was expressed in Sherman Bourquin units

purified carbohydrates (sweets), and fat As is the case with all vitamins, diets can be arranged so that protection is secured by only one or two items, but such diets are apt to be deficient in other respects and a liberal well diversified diet is the best The relatively large amounts of nicotinic acid in even a moderately 'good' diet the stability of this factor to heat and other processes of preserving and cooking and the apparently good storage make this deficiency unlikely to occur and easy to prevent except under rather extreme circumstances Such a conclusion agrees well with the known facts regarding the incidence and epidemiology of the disease That it occurs in endemic form in certain large groups of the population is less a measure of the general availability of the protective factor than an indication of the severity of the economic ills which make even a moderately good diet impossible in the group among which the disease occurs endemically

Protection Special protection, often requiring supplements in addition to the diet, is indicated for those who cannot obtain proper food and in those cases in which other disease interferes with the intake and absorption of food Such supplement in addition to diet is best provided by concentrates such as brewers yeast which will supply not only nicotinic acid but other vitamins which are also apt to be deficient Only occasionally or when for some reason concentrates cannot be taken will it be necessary to use pure nicotinic acid or amide Doses of 30 Gm (one ounce) or more daily, depending on the amount available in the diet, of a good grade of dried brewers yeast or similar preparation will suffice to protect against the development of a deficiency In those cases in which severe limitations of intake or interference with absorption exist as in patients maintained on parenteral feeding, or in the insane, delirious or severely alcoholic nicotinic acid itself may be used though in such cases supplements of the other necessary food factors will be required in addition For pur

diet should contain liberal amounts of milk, animal pro tein including liver if possible, eggs, and a considerable proportion of fresh and raw vegetables and fruits Some difficulty may be encountered in securing the intake of a complete diet at first but as the patient's general symptoms improve, the mouth lesions clear, and the appetite returns no difficulty will ordinarily be encountered, aside from an economic one, in securing an adequate intake of natural food stuffs

When adequate doses of nicotinic acid are given fol lowed or accompanied by a concentrate such as yeast, and a liberal, adequate diet, the administration of nicotinic acid can usually be stopped in a few days and the patient continued on the concentrate for a few weeks after which the diet is sufficient provided it is taken In patients who for one reason or another cannot or will not take an ade quate diet nicotinic acid even in increasing doses may fail to prevent recurrences There is some evidence that in these cases the addition of riboflavin will make the smaller doses of nicotinic acid effective and prevent relapses Or dinarily, this is provided by the yeast or similar concentrate but at times it may be advisable, particularly in the begin ning, to give pure riboflavin This can be given in doses of 3 to 5 mg per day by mouth and is available in a variety of preparations Riboflavin is available in 5 mg tablets or in powder It is very unstable to light and is rather rapidly destroyed in solution For this reason the tablets are pre ferred If desired the powder may be weighed out in proper doses, placed dry in gelatin capsules and kept in a box away from the light

In addition to nicotinic acid and some of the more ob scure factors of the B complex which may be concerned in pellagra, other deficiencies are often present These in clude thiamin (vitamin B_1), ascorbic acid (vitamin C), pro tein, and iron A deficiency of thiamin (B_1) is common and is responsible for the peripheral neuritis which is frequently

found in pellagrins, especially in the sporadic cases asso
ciated with chronic alcoholism If mild this deficiency is
relieved by such concentrates as yeast and by the diet In
many cases, however the deficiency is sufficiently severe or
advanced to require supplements of the pure vitamin In
this case thiamin may be given in doses of ten milligrams
a day until improvement occurs In severe cases larger
doses may be given and administered intramuscularly or
intravenously (See Chapter 3) Deficiencies of ascorbic acid
(vitamin C) and iron may be treated in the same way (see
Chapters 6 and 11) Protein deficiency is ordinarily treated
by liberal intake of food high in protein but in severe cases
and especially in those in whom chronic or repeated pel
lagra may have caused difficulty in absorption, transfu
sions may be given to secure quicker results

Few of the manifestations of pellagra will require treat
ment other than that herein outlined The dermatitis how
ever is an exception Simple erythema responds readily and
even more severe dermatitis with blebs and superficial
ulceration and crusting will heal readily, though the time for
duration of complete healing is longer because of the require
ment of desquamation and anatomic repair Where there
is secondary infection local treatment may be required
temporarily For this purpose wet dressings of saline and
a protective covering will usually suffice A mild antiseptic
dressing may be necessary but in general the less irritating
the applications the better During the first hours of treat
ment a sedative may be necessary and restraint may be re
quired Treatment of complicating diseases is, of course,
important and may have an important bearing on the re
sponse and cure of the pellagra

Latent or subclinical cases can be handled by diet alone
or with the addition of a concentrate such as yeast which
will help by supplying thiamin to increase the appetite and
thus secure an adequate intake of food Even in these cases,
however, nicotinic acid may be employed to advantage

at the begınnıng when they serve both as a therapeutıc test of the dıagnosıs and as a means of quıckenıng the re covery For thıs purpose, doses of 50 to 150 mg per day ın dıvided doses wıll be suffıcıent In these cases, however, as ın all others the ultımate aım ıs to secure the ıntake of a proper amount of food and every effort to accomplısh thıs must be made by educatıon of the patıent and all other means Faılure to do so means recurrences

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5

Riboflavin Deficiency

(Ariboflavinosis—Hyporiboflavinosis)

HISTORY

THE characteristic clinical expression of riboflavin deficiency was first described by Sebrell and Butler in 1938[1] It consists of a cheilosis, a stomatitis at the angles of the mouth, a seborrheic dermatitis a glossitis Later Sydenstricker, Sebrell Cleckley and Kruse added vascularization of the cornea The lesions of the lips and mouth have been familiar to clinicians for years but their relation to a vitamin deficiency was unknown until it was demonstrated by Sebrell and Butler The lesions are commonly found in pellagra in which there is frequently an associated riboflavin deficiency

NATURE AND FUNCTION

Riboflavin,* known also as lactoflavin vitamin B_2 or vitamin G, is a complex water soluble pigment with a greenish yellow fluorescence It is found widely distributed in plant and animal tissues and may be a part of every living cell The flavins have been known for many years and have been isolated from many sources such as milk, eggs, liver, yeast, et cetera Before the essentially single nature of riboflavin was known the pigment was designated according

* Riboflavin is 6 7-dimethyl-9 (di ribityl) isoalloxazine with the following structural formula Other flavins and flavin derivatives have been

to its source as lactoflavin, ovo flavin, hepato flavin, et cetera Later the flavins were recognized as a part of Warburg's yellow ferment and about the same time its growth promoting and vitamin like properties were recognized It has since been isolated in pure form, synthesized, and its various physical and chemical properties fully determined One of the most characteristic properties is its fluorescence—a property utilized in tests for its presence and concentration

Riboflavin is water soluble, relatively stable to mineral acids and oxidizing agents, but sensitive to alcohol and easily decomposed by light In the body riboflavin forms a phosphoric acid ester which is combined with a protein to form an oxidation enzyme, the yellow oxidation enzyme (yellow ferment) of Warburg and Christian As such an enzyme it acts as a hydrogen carrier in the cell Thus it is a part of the oxidation reduction mechanism of the body which we encountered in the case of thiamin and nicotinic acid Although much is known of the chemistry of riboflavin and its function in oxidation systems, little or nothing

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synthesized and some possess vitamin activity but none more than riboflavin and probably all have less In the body it forms a coenzyme system but also occurs free and combined with phosphoric acid

CH_2 OH
H C OH
H C OH
HC OH
CH_2
H C N N
CH_3 C C C CO
C C C
CH_3 C N C

is known of the manner in which a deficiency produces the pathologic changes which are observed clinically The mechanism of the functions of the various coenzymes, in cluding that in which riboflavin is concerned, are so com plex that for the present it is impossible to separate with assurance the disorders of metabolism which result from defects in anyone of the various components of the complex system and assign to it a characteristic disorder in the clinical sense However it is felt that adequate amounts of riboflavin are essential for the transport of hydrogen in the cells And it has been suggested that, in avascular structures such as the cornea, a deficiency of riboflavin prevents this trans port and initiates vascularization as a compensatory pro cess Hence the development of the characteristic lesions in the eye in this deficiency In any event a deficiency of ribo flavin produces a characteristic clinical disorder distinct from any part it may play in association with other vitamins Evidence for this is available both in animals and in man Until recently, and only after the discovery of the role of nicotinic acid riboflavin had been rather generally and prematurely considered the anti pellagra substance in spite of considerable experimental and clinical evidence to the contrary This error has led to much confusion in both the experimental and clinical fields For instance the pellagra preventive value of many foods was based on their vitamin G (riboflavin) content as determined by the rat growth method rather than on their true pellagra preventive value which may differ greatly

Relatively little is known of the absorption storage, and excretion of riboflavin in man Presumably it is widely dis tributed in the cells It has been found in the eye of man and is present in mother s milk Rather large amounts are ex creted in the urine 0 5 0 8 mg daily and in the stools, and this is increased if the amount in the diet is greatly

augmented In certain instances excretion fails to parallel large intakes suggesting storage though no large depots are known When intake is greatly reduced, excretion continues in reduced amounts The daily requirement is not known with exactness but is estimated to be about 1 2 5 milligrams varying with the calories No toxic effects have been reported

PATHOLOGY AND PATHOGENESIS

Little is known of the pathologic changes produced by riboflavin other than the gross appearance of the lesions of the mucus membranes and the skin and the microscopic changes in the eye as seen with the slit lamp The latter are described under Symptoms and Signs Histologic studies of the characteristic lip and skin lesions have not been reported Very likely they will prove no more specific than the cellular changes found in pellagra to which they appear quite similar

INCIDENCE AND EPIDEMIOLOGY

Little is known of the actual incidence of the deficiency, particularly if one includes the mild or subclinical cases It appears now to be a common accompaniment of pellagra and it is likely that it has about the same incidence as that disease as far as cases of frank deficiency are concerned The conditions of dietary error associated with the two deficiencies are similar and it would be expected that the two would occur with much the same frequency However, a startlingly high incidence has been intimated even among individuals whose diets presumably are satisfactory and who are not known to be suffering from other disease Recent observations suggesting that slight degrees of corneal vascularization may not be evidence of a general riboflavin deficiency may explain some of this apparent frequency More exact statement as to incidence must await further study

SYMPTOMS AND SIGNS

The symptoms of riboflavin deficiency are closely asso ciated with the characteristic lesions which are the inflamma tion of the lips fissures (rhagades) at the corners of the mouth, glossitis a dermatitis, and a vascularizing keratitis The ocular symptoms are the most constant and may appear before other symptoms of the deficiency They consist of photophobia, burning, itching and irritation' of the eyes visual fatigue, and dimness of vision which is not relieved by the correction of refractive errors With these ocular symp toms the physical changes in the eye occur which are char acteristic and may appear before the other lesions The earliest and commonest change is a circumcorneal injection This may be visible grossly but is better seen on examination with the slit lamp as a congestion and proliferation of the limbic plexus Accompanying this there are a great number of narrow capillary loops at the edge of the scleral digita tions, obliterating the normal avascular zone between the plexus and the sclero corneal junction The next stage is an actual invasion of the cornea by small capillaries arising from the apices of the scleral loops They are best seen in the nasal and inferior quadrants and lie just below the epithel ium As the lesion proceeds a progressive series of anasto motic capillary loops and projections is formed until an extensive superficial vascularization takes place As the le sions progress, deeper invasion occurs until finally general vascularization develops The superficial vascularization is more intense than the posterior in contrast to the greater posterior vascularization in syphilitic interstitial keratitis With the greater degrees of vascularization opacities occur Superficial nebulae first appear as slight steaminess and later interstitial nebulae occur Superficial punctate opaci

ties and posterior opacities are less common Changes in the iris occur with a frank iritis in some cases but more often there is only congestion with accumulation of pigment on the anterior surface The latter may be difficult to distinguish from variations in the appearance of the normal iris but may be detected by the changes following treatment There is a mydriasis in some cases

The symptoms and objective changes in the eyes disappear rapidly under treatment Within 48 hours photophobia, burning and itching have usually disappeared and there is lessened circumcorneal injection The acuity of vision is soon restored In early cases the invading capillary loops have been observed to empty in as short a time as 24 hours, more severe cases require several days Superficial opacities clear sooner than the interstitial nebulae and the posterior opacities disappear last of all With the clearing of these lesions there is a resolution of the changes in the iris

If relapse occurs because of interruption of treatment or for any other cause there is a prompt return of symptoms, photophobia appearing first and within a few days Objective changes are visible with the slit lamp

Burning and soreness of the lips and tongue and soreness in the corners of the mouth accompany the oral and lingual lesions Burning of the tongue may be a particularly troublesome symptom The discomfort may be great enough to interfere with eating and the tongue may be so sore that swallowing is difficult The lesions on the lips begin with a redness of the vermilion border of the lips, the latter area at this stage being covered with a thin grayish coat which later desquamates leaving the lips redder than before In severe cases there may be a shallow ulceration and crusting With this, or independently, there are shallow radiating fissures (rhagades) at the corners of the mouth, slightly red often covered with a slightly yellowish greasy exudate which

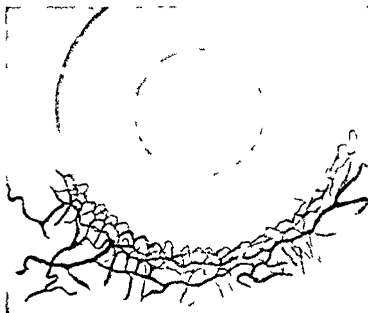


FIG 6 Diagram indicating the grade of congestion and proliferation of the limbic plexus This is found in the earliest stage of ariboflavinosis (*Figures 6 7 8 and 9 are here reproduced by courtesy of Dr V P Sydenstricker and the Journal of the American Medical Association*)

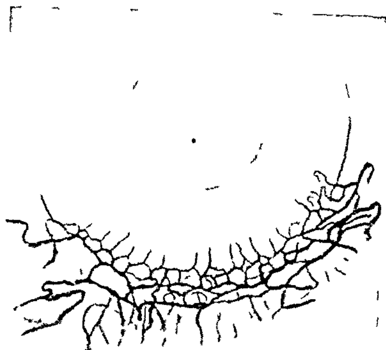


FIG 8 Further invasion of the cornea by the capillaries and increased vascularization

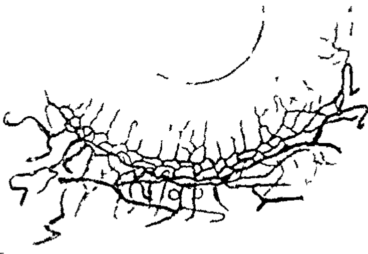


FIG 9 More marked invasion and vascularization in the cornea

may be removed leaving a raw but non bleeding surface Healing may leave shallow linear scars In some cases there may be a fine, scaly, seborrheic dermatitis about the nose in the nasolabial and nasomalar folds and on the chin and malar prominences Sometimes in the latter area the lesion has a butterfly outline Follicular keratosis of the cheeks, forehead and chin have been described

The glossitis is characteristic but is very apt to be confused with that of pellagra with which it is often associated It is best observed after a pellagrous glossitis has been cured leaving the distinct and characteristic glossitis of riboflavin deficiency The tongue is a purplish or magenta red, with a clean surface, often fissured The papillae are large and flat and there is no true atrophy of them In some cases the borders of the lips seem to have the same peculiar magenta hue

Sydenstricker et al [2] have described a dermatitis of the hands (without the involvement of the feet) quite different from that of pellagra from which their patients had suffered previously Whether a dermatitis in other areas than the face and lips may be due to riboflavin deficiency is as yet unknown

Lesions such as have been described above have been observed for years, in association with pellagra and independently, and have been described under such terms as marginal stomatitis (Stannus) When encountered in pellagra they have been considered as an accessory lesion of that disease Whether other manifestations of the pellagra syndrome may be due to riboflavin deficiency cannot be determined at present any more than whether riboflavin deficiency is an essential part of that clinical disorder For the present it seems best to consider pellagra as a disease entity to which there may be added at times the specific expression of riboflavin deficiency

circumstances The doses described in the chapter or lagra will be adequate in most cases Only occasionally it be necessary to employ the pure vitamins

Riboflavin is rather widely distributed in foods T especially rich in it are milk, liver, eggs, green vegeta and fruits

Curative treatment likewise is easily accomplished in cases by diet alone or with the added use of concent such as yeast and wheat germ In more severe cases, ever, and if the intake or absorption of food is greatl stricted, treatment with the pure vitamin may be neces Doses of 3 to 5 mg a day are usually sufficient In s cases considerably larger doses are necessary, 10 to 30 a day In some cases it may be necessary to give the vita parenterally, particularly since some patients seem to difficulty in utilization as well as in absorption from gastrointestinal tract For this purpose it may be give doses of 10 to 50 mg hypodermically or intraveno Usually the smaller doses are effective

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Ascorbic-Acid (Vitamin-C) Deficiency

(Scurvy—Infantile Scurvy or Barlow's Disease—Hypovitaminosis C)

HISTORY

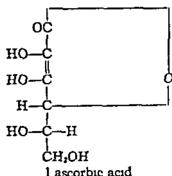
THE CLINICAL expression of severe vitamin C deficiency is scurvy, a disease characterized by weakness, anemia, swelling of the gums, and multiple hemorrhages In its classical form it has been known for centuries and recognized as likely to occur among soldiers and sailors inmates of prisons and institutions and those suffering from poverty and exposure As a result of Lind's classical observations among the crews of sailing vessels it was one of the first diseases to be recognized as due to lack of some food factor and preventable by supplying the missing substance Infantile scurvy identical in its essential etiology and pathogenesis to scurvy in adults was unrecognized until the studies of Cheadle and Barlow in the 19th century, due to the fact that the clinical manifestations were modified by factors of growth and development In recent years actual scurvy is uncommon in either adults or children though there appears to be an increase in its incidence recently in certain groups of the population whose economic status has been lowered There is, however a large amount of hypovitaminosis C, a subclinical or latent form of scurvy which presents few or none of the characteristic signs and symptoms and is detectable only by vague and nonspecific symptoms and signs by a knowledge of an inadequate intake, and by laboratory methods

NATURE AND FUNCTION

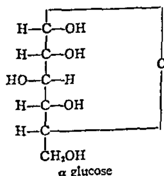
Vitamin C is ascorbic (cevitamic) acid, a six carbon chain acid related to the hexuronic acids which in turn are derivatives of the simple sugars * It is a powerful oxidation reduction agent, that is to say a substance capable of reducing many organic compounds during which process it is reversibly oxidized to dehydroascorbic acid Dehydroascorbic acid in turn is readily reduced back to ascorbic acid or vitamin C by oxidation processes in the body Oxidation is also accomplished by various metals, particularly copper, and other inorganic substances In the body it is active only in the reversibly oxidized or reduced form When irreversibly oxidized it is no longer biologically active

Vitamin C cannot be synthesized by the human body and its entire supply is normally obtained from the food There is a considerable difference among species in this respect Some animals such as the chicken, rat, and dog are able to synthesize it, others cannot The guinea pig is like man in

* Vitamin C or ascorbic acid is 2 3 enediol 4 lactone with the following structural formula Two forms (isomers) exist l ascorbic acid and d ascorbic acid Only the l form is physiologically active



l ascorbic acid



α glucose

Its close relation to glucose is shown by the formula for glucose which is given for comparison A number of related compounds with similar chemical properties have been prepared some of which show slight degrees of antiscorbutic activity None are of practical importance at present

this respect, a fact which explains its suitability and frequent use as an experimental animal in studies of this vitamin

Vitamin C is rapidly and completely absorbed in the intestine It is distributed throughout the body but is found in higher concentrations in certain organs and tissues than elsewhere Structures rich in vitamin C are the adrenal glands, pituitary gland, and intestinal wall It is unlikely that greater concentrations in these organs represent storage depots Instead it may reflect a higher level of metabolic activity in those sites or a peculiarity of functioning requiring larger supplies *The vitamin is found in the circulating* blood in concentrations which apparently reflect, except for fluctuations caused by immediate variations in intake and absorption, the general level of the vitamin in the tissues An exception to this general statement is the fact that sharp restriction or complete lack of intake is followed in a short time by a drop in the concentration in the blood to a rather low level irrespective of the fact that relatively large stores may still be present in the tissues This fact has a bearing on the use of concentration in the blood as a diagnostic test and suggests a tendency to restrict the loss of the vitamin from the body when the intake is reduced, similar to the drop in nitrogen excretion which occurs when the intake of protein is reduced

Under conditions of fasting there is more vitamin C in the blood cells than the serum The amount in the white cells is much greater than in the red cells but of course the mass of white cells is less An excess in the serum may occur following the intake of vitamin C and before a balance has been established between the cells and serum Fluctuation in both plasma and serum is greater than in whole blood The latter represents better the state of the body with respect to vitamin C metabolism but technically, determinations on serum or plasma are to be preferred for clinical use

Under ordinary conditions there appears to be little loss

of vitamin C in the stools It is possible that destruction of the vitamin may occur in the intestine thus accounting for the failure to recover it in the feces Certain organisms have been reported to cause considerable destruction of the vitamins These, however, are not ordinary inhabitants of the intestine and conclusive evidence is lacking that this mechanism is a common or even actual cause of failure of proper supply Excretion and storage studies suggest that utilization loss is insignificant under most circumstances

Vitamin C is found in the cerebrospinal fluid in a concentration somewhat greater than in the blood, and is also found in the tears and perspiration It is secreted in the milk in both those species which synthesize it and those which must depend on a dietary intake In the latter, the amount in the milk depends on that in the diet This is the case in man and it is of interest in this connection that human mother s milk, if she is on a proper diet, is several times richer in vitamin C than cow s milk

Although, as indicated above, vitamin C may be stored to some extent, its storage appears to be less than is the case with some of the other vitamins In guinea pigs a complete absence of vitamin C from the diet is followed by histologic evidence of the deficiency in as short a time as a week Storage, saturation, and excretion studies in man as well as clinical experience indicate a similar behavior in humans A complete lack of the vitamin in the diet is not common, however, in human subjects

In addition to that portion utilized, and the wear and tear loss of the vitamin, there is a constant but variable loss in the urine Vitamin C is excreted by glomerular filtration and is resorbed by the tubules There is a maximum rate at which the tubules can resorb it and if this is exceeded the excess appears in the urine Under conditions of normal intake the excretion in the urine is an expression of this excess However, with a low intake, low stores, and a low

concentration in the plasma, a certain amount is still ex creted daily in the urine and continues to be excreted as long as there is any in the blood Even in the presence of mani fest signs of scurvy the excretion seldom falls to zero

Vitamin C has been isolated and synthesized in pure crys talline form Although vitamin C is very easily oxidized the dry crystals are stable to air and daylight over a period of several years In aqueous solution it is very sensitive to light under ordinary conditions However in aqueous solution with an acid reaction (pH below 7 0), and protected against such catalytic agents as traces of copper, et cetera, it is quite stable and it is not auto oxidizable within the normal pH range of animal and vegetable tissues

Prolonged cooking and drying storing and aging destroys vitamin C in foods Copper catalyses such an oxidation and even traces of this metal greatly increase the loss Under proper conditions pasteurization causes insignificant losses but, in general, cooking and storing are apt to destroy a con siderable portion The loss is much greater in alkaline solu tions and one of the advantages of the citrus fruits as a source of the vitamin is the protective effect of their acid reaction

Although a great deal is known about the chemical prop erties of vitamin C and its biochemical activity as an oxida tion reduction agent, almost nothing is known of the way in which those activities accomplish the function of vitamin C in the body Vitamin C is necessary for the maintenance of the normal structure and proper formation of the inter cellular ground substance of mesothelial tissue or structures derived from this tissue such as connective tissue, bone, teeth, capillaries, et cetera In the intercellular ground sub stance are the collagen bundles Vitamin C appears to con trol the formation of this collagen although it is not known whether it does so through its action in the cells themselves or by acting on the colloids of the intercellular substance

directly In the absence of an adequate supply of the vitamin the intercellular ground substance loses its normal character and the collagen bundles disappear New intercellular sub stance is not laid down With a partially deficient supply an imperfect or defective ground substance may be found, with greater deficiency none is found, and that previously present disappears As a result the pathologic changes typical of the disease appear

PATHOLOGY AND PATHOGENESIS

The disappearance or defective formation of proper inter cellular ground substance (lack of collagen fibers and bundles in tissue of mesenchymal origin) is the fundamental pathologic lesion of scurvy and vitamin C deficiency This *lesion and the kind of tissue affected* (capillaries and con nective tissue especially) explain the nature and widespread distribution of the lesions The histologic appearance is easily recognizable and has been well studied In addition to the general lesions, its effect on particular structures and tissues account for the characteristic gross lesions These are found in association with the capillaries, bone, teeth, and gums

The capillary lesions are unique in that the exact loca *tion of the defect is unknown* Although the endothelial cells are believed to be bound together with a cement sub stance, this has not been demonstrated Hence morphologic changes cannot be demonstrated The defect may lie in the thin connective tissue and collagen bundles that surround the vessels In any event the capillary wall becomes defective and loss of blood (hemorrhage) occurs

The skeletal changes are among the most constant features of scurvy in children and the histologic changes in growing bone constitute the classical lesion of infantile scurvy Bone growth slackens and finally stops The regions most often

affected are the costochondral junctions the distal end of the femur, and proximal end of the tibia and humerus The lesions both gross and microscopic, while characteristic and generally easily recognizable may be simulated by other disease, such as rickets and may be difficult to detect when that disease complicates the scorbutic lesion Histologically one of the earliest changes is an alteration in the osteoblasts The osteoblasts lose their shape, leave the trabeculae tend to resemble fibroblasts, and may produce collagen and fibrils (Dalldorf) Irregularities develop in the columns of cells in the proliferative cartilage and at the cartilage shaft junction, the normal lattice of calcified cartilage being replaced by irregular masses of calcified cartilage in fibrous tissue Until growth ceases entirely, these are pushed forward and piled up in an irregular fashion in an area of the shaft normally occupied by marrow tissue Thus the normal lattice is widened and irregular This matrix seems brittle and micro scopic fractures appear leading to displacement of fragments and a disorganization of the lattice '

At the same time osteoblasts which are often atypical in appearance fail to form new bone and new trabeculae Thus between the older trabeculae previously formed and the calcified matrix, there is a zone free of trabeculae constitut ing the zone of rarefaction This is essentially a zone of fibrous tissue, hence an area of lessened resistance to stress and the zone through which the subepiphyseal infractions' *or epiphyseal separation occur*

While bone formation ceases bone resorption continues perhaps at an accelerated rate so that there is a general thinning or atrophy throughout the whole bone including the cortex The latter is particularly thinned next to the cartilage The bone often becomes so thin that it is less strong than the cartilage

Changes in the marrow occur, the normal hematopoietic tissue being replaced at the epiphyseal end by so called



Fig 10 Bone changes due to scurvy Note the clefts and nicks at the metaphysis caused by hemorrhage and the thin cortices and generalized rarefaction of the bones

affected There is hyperemia and edema of the pulp fol lowed by atrophy and degeneration of the odontoblast layer Hemorrhage into the pulp may occur with the formation of cysts, areas of calcification, or actual osteoid tissue The teeth are loosened due to rarefaction of the alveolar processes and may be lost Cure can be effected but histologic evidence of the attack always remains Relatively few studies have been made of the teeth of children with scurvy but there is good reason to believe that changes occur similar to those found in adults Normal dentition is almost certainly interfered with and defective formation of the teeth probably occurs The opinion is still divided as to the relation of vitamin C deficiency to caries but there is increasing evidence that it is a factor and probably an important one

The gums are affected characteristically only when there are teeth Thus infants and toothless persons may fail to show one of the typical lesions Lesions of a sort do occur however and a certain number of infants will exhibit swell ing redness and other changes in the gum over unerupted teeth Hemorrhage of the gums may occur just as from any mucous membrane surface The characteristic gingivitis begins with hyperemia and redness of the papillae followed by swelling hemorrhage retraction ulceration and sec ondary infection For additional changes see Diagnosis

The lesions of the capillaries cause hemorrhages in many parts of the body Those in the skin are very frequent and because of their visibility are important in the diagnosis The most characteristic lesion is a petechial perifollicular hemorrhage but there may be large ecchymoses and suggilla tions Some variation in the incidence of hemorrhages in the skin occurs Infants have them less frequently and they are less severe in some individuals than in others with the same degree of deficiency Other changes in the skin such as hyperkeratosis often associated with perifollicular hemor rhage are probably due to other deficiencies In addition

to cutaneous hemorrhages, deep hemorrhages occur sub cutaneously or intramuscularly The deeper ones often extend along fascial planes They are much more common in the lower extremities Subperiosteal hemorrhages described under skeletal lesions are uncommon in adults but may occur There may also be hemorrhages into the various structures of the eye or within the orbit, hemorrhagic effusions in the serous cavities, and much less often bleeding into the gastrointestinal tract Epistaxis is not uncommon The weakening of the capillaries causes an edema which appears early, and may appear in the legs in even mild cases Pericardial, peritoneal, and pleural effusions (transudates) occur in that order of frequency

In addition to these well known pathologic changes, advanced cases may show severe degenerative changes in the skeletal muscles, cardiac hypertrophy, atrophy of the bone marrow with anemia, and atrophy of the adrenals Calcification of hemorrhagic areas occurs in advanced cases

As Dalldorf has pointed out the factors of growth and stress are important in influencing the type and location of the lesions in scurvy For this reason the most severe bone lesions occur in the young growing child Adults show little more than subperiosteal hemorrhage, and this *only* in the more severe cases The sites of greatest growth or metabolic activity are most affected, and these sites vary at different ages Normally trauma plays a part in the location of skin hemorrhages the lower extremities with their greater venous pressure are especially susceptible The pressure of clothing or the use of certain muscles may be determining factors

The lesions just described are, however, those of advanced deficiency, i e , of actual scurvy In the latent or subclinical cases, i e , hypovitaminosis C, many or most of them are lacking and only by histologic examination, or under spe

cial circumstances and in special sites can their presence be detected Only by reference to animal experimentation and occasional postmortem studies are these minor changes of slight deficiency related to the clinical picture of latent or subclinical scurvy *Yet it is this type of deficiency which at the present time is of greatest importance because of its much greater frequency and insidious effects* Even the milder deficiencies cause structural and functional changes and even if these changes are not accompanied by gross morphologic lesions they are nevertheless important Vitamin C has an essential role in general growth processes which must be affected by even limited deficiencies Reparative processes, particularly those concerned with connective tissue, are affected Slight grades of vitamin C deficiency interfere with wound healing and it is probable that many cases of improper healing and rupture of surgical incisions are the result of such deficiencies This is particularly important in patients with gastrointestinal disease since they frequently require operative treatment and are also quite likely to be deficient in vitamin C Clinical experience has demonstrated a high incidence of infectious disease in scurvy and although a specific relationship of vitamin C to infections has not been demonstrated, it is very probable that even mild deficiencies exert an influence on the course of an infection if not on its actual occurrence Local infections pneumonia, diphtheria, and bacillary dysenteries are complications found to be common in scurvy

The repair of the soft tissue lesions caused by vitamin C deficiency is very rapid Adequate treatment is followed in twenty four hours by signs of regeneration of proper connective tissue Fibroblasts put out collagen fibrils and capillary buds invade bloodclots There is no record in the literature of pathologic changes due to excessive amounts of vitamin C

INCIDENCE AND EPIDEMIOLOGY

The exact incidence of vitamin C deficiency is unknown Frank scurvy is uncommon in adults, and due to the more general use of antiscorbutics (orange juice, et cetera) in the feeding of infants it is becoming uncommon in them Recently there appears to have been an increase in the incidence of scurvy coinciding with the general lowering of income levels It is interesting that the greater increase in incidence is found among those who have dropped from a previously satisfactory economic status rather than in those permanently in the lower levels In spite of this increase frank scurvy is infrequent

On the other hand subclinical scurvy or hypovitaminosis C appears to be a fairly common condition Recent studies in as widely separated regions as the North East, and the South Central states have reported an incidence as great as 30 or 40 per cent in groups of persons whose diets were believed to be fairly satisfactory Some doubt may be expressed as to the correctness of those conclusions because of differences of opinion as to what constitutes hypovitaminosis C and the interpretation of the tests which have been employed Nevertheless, there is increasing evidence to indicate that a relatively large number of persons have hypovitaminosis C or subclinical scurvy and that these include individuals whose diets are generally considered satisfactory

As is the case with the deficiency of other food factors vitamin C deficiency may be considered to occur in endemic epidemic and sporadic forms In the endemic and epidemic types the deficiency is due to an inability to secure enough of the vitamin in the diet because of factors common to a group Sporadic cases are the result of individual factors such as inability to obtain proper food, ignorance as to what constitutes a proper diet, or improper methods of storage,

handling and preparation of food The latter is an impor tant factor in both endemic and sporadic forms because of the easy destructibility of the vitamin Interference with ab sorption is much less of a factor than is the case with other vitamins but many conditions, including the effects of other disease, may affect the intake in an individual There is also reason to believe that certain diseases, infections, fevers, and those associated with increased metabolism, increase the need for vitamin C or cause an increased destruction and make an otherwise normal intake inadequate The new born infant s store depends on the nutrition of the mother and in any case is rapidly depleted after birth An artificially fed infant is liable to develop a deficiency unless supplements are provided and breast fed infants may suffer if the mother s nutrition is inadequate Poor hygiene, overcrowd ing dampness cold, and physical work favor the develop ment of a shortage Obviously any of the factors peculiar to the individual may affect a member of a group already de ficient and exaggerate the deficiency

SYMPTOMS AND SIGNS

The signs and symptoms of scurvy are well known In the adult the classical picture is that of an afebrile illness with an onset characterized by a few days of lassitude and weak ness and muscle and joint pains Subsequently petechial hemorrhages in the skin swelling and tenderness of the legs swelling and bleeding of the gums palpitation and slight shortness of breath appear These earlier symptoms are in turn followed by greater swelling larger hemorrhages in various sites effusion into the pleura and pericardium, pallor due to edema and anemia greater mental depression and apathy The teeth become loosened and may fall out and infection and ulceration of the gums develop Epistaxis and in severe cases bleeding from stomach bowel or genito

urinary tract occurs Painful subcutaneous hemorrhage occurs which may be followed by infection and ulceration Death may occur from hemorrhage or shock but is most often due to intercurrent infections, particularly bronchopneumonia

In infants many of these manifestations may be lacking even with severe deficiency The symptoms and signs are related mainly to the long bones There is pain, particularly of the legs, and this is followed by swelling Frequently the pain is sufficient to cause immobility and simulate paralysis particularly *in the adult Tenderness in the legs is by far the* most frequent symptom in infants The ends of the bones may enlarge as in rickets and in advanced cases there may be separations of the epiphyses or even fractures A common site for enlargement (and tenderness) is the costochondral junction where a rosary similar to that seen in rickets may form Tenderness is not necessarily confined to areas of hemorrhage Disturbance in general health is manifested by pallor, failure to gain weight (edema may mask this) or loss of weight, weakness, and irritability Fever is common in infants with rapid shallow breathing (due to changes in the ribs) and a rapid pulse In some cases there is vomiting and diarrhea Occasionally other less common symptoms such as melena, hematemesis, or protrusion of an eye due to hemorrhage may be the presenting symptoms

If the teeth have not erupted the gums may show no changes but often, over teeth soon to erupt, the gums will be swollen, dusky red and tender (frequently falsely attributed to 'teething) If teeth are present there will be the typical gingivitis Subcutaneous hemorrhages are less common than in adults but purpuric spots are likely to be present, especially in areas of pressure or trauma as on the buttocks, on the face and neck (from crying), where the clothes bind, et cetera Occasionally a large cutaneous hemor



Fig 12 Bone changes due to scurvy after treatment for nine days Note the subperiosteal hemorrhages (calcifying) healing of the metaphyseal lesions and the rings of *increased calcification* about the centers of ossification (Wimberger's rings)

laboratory tests including the x ray Although a diagnosis by history and clinical signs may be made easily in frank cases of scurvy, these means are of little assistance in the much more numerous cases of latent or subclinical scurvy or hypovitaminosis C The changes seen in x ray pictures are characteristic when present but are absent or confusingly indefinite in early cases, especially in older children and adults For the diagnosis of the milder or earlier deficiencies laboratory tests are needed though the diagnosis may be sug gested by indefinite symptoms, and knowledge of a de ficient intake or absorption Fortunately, the clinical labora tory tests are quite simple

Laboratory tests and special procedures for the diagnosis of hypovitaminosis C include the following 1 a deter mination of vitamin C excretion in the urine, 2 a deter mination of the vitamin C concentration in the blood 3 "load' or ' saturation ' tests, 4 the capillary resistance test, 5 the x ray, and 6 biomicroscopy of the gums

Excretion Tests A number of chemical tests have been devised for the estimation of vitamin C (ascorbic acid), and most of them can be applied to the urine However, the one which has been most widely used and is most simple and re liable is the test using the dye dichlorophenolindophenol This test is well suited for clinical use and is the one de scribed here * Dichlorophenolindophenol is decolorized by vitamin C and in practice a standard solution of the dye is titrated against an unknown specimen of urine Although the reaction is not strictly specific for vitamin C, the dye being reduced by other substances, the circumstances are such that reduction due to vitamin C and that due to other agents can be distinguished under certain conditions and when properly performed the test is reasonably specific for vitamin C By this means the amount of vitamin C in urine can be quickly and easily determined Testing of single

specimens is of little value Twenty four hour specimens however level out variations in single specimens and reflect with fair accuracy the status of the body with respect to vita min C nutrition

Objections have been raised to the determination of the amount of the twenty four hour urinary excretion of ascor bic acid as a diagnostic test The major objections have been that normally there is a large variation in the amount of ascorbic acid excreted daily in the urine, and that tem porary variations in the dietary intake cause rapid decreases in the amount of the vitamin excreted in the urine Hence the objection may be made that low values are found with out clinical evidence of the deficiency As a matter of fact, the twenty four hour excretion is a fairly good measure of vitamin C nutrition if the results are interpreted in the light of other clinical evidence as should be the case in all such tests It must be remembered that although a rather wide range of excretion is found normally there is a lower level of excretion below which persons on a reasonably nor mal intake of vitamin C seldom fall Excretion below this level is most often found associated with an intake low enough to indicate a probable deficiency Also, when fluc tuations in excretion are found in association with changes in intake these fluctuations occur at the level and within the range of normal output The significance of this will be made more apparent by a consideration of the load or saturation test which is generally recommended by those who feel the daily excretion is unreliable It is known from saturation tests that when a deficiency of the vitamin exists reasonable amounts of the vitamin may be given and will not appear in the urine, and hence will not significantly affect the excretion for that day It is apparent, therefore, that daily excretions below the lower level of normal excretion, are unlikely to be influenced by such daily varia tions as are encountered in the diet The criticism that there

is frequently a lack of signs or symptoms at low levels of excretion applies to all tests of preclinical deficiency This criticism can be answered only by the experimental and clinical evidence showing that pathologic processes, both functional and structural, do exist before gross physical signs appear

Properly performed then, the twenty four hour excretion gives reliable diagnostic evidence of deficiency if properly interpreted Standard values have not yet been generally established and accepted Single tests should not be considered conclusive and low values should be checked by repeated tests or by a "saturation test The daily excretion is a poor gauge of the severity of the deficiency since, as has been pointed out, some vitamin may still be excreted by patients with scurvy This is better measured by the load or saturation test Standard values have not yet been generally established and accepted Most adults on the usual 'good diet excrete from 20 to 50 mg daily Values below 15 mg are suggestive of a deficiency and should be investigated further Normal children excrete 10 20 mg daily dependent on the intake of vitamin C There is reason to believe that these values are minimum and that with a more nearly optimum intake most persons would excrete the larger amounts

Concentration of Ascorbic Acid (Vitamin C) in the Blood * *This test has the advantage of avoiding the collection of twenty four hour urine specimens but the technic is somewhat more difficult and requires more time It can however, be used in subjects from whom twenty four hour urine specimens cannot be obtained It has the disadvantage that the results are quite easily influenced by temporary variations in vitamin C intake In particular low intake for a few days is apt to be followed by a considerable drop in the blood ascorbic acid though the body stores are as yet but

* The method for the determination of the ascorbic acid in the blood is

little depleted Similarly the ingestion of a good sized amount will raise the blood level temporarily Therefore the blood sample should be drawn in the fasting state or at least several hours after an ordinary meal and results should be inter preted in relation to the diet Determination may be made on serum or plasma However, most of the clinical studies have been made with serum or plasma

Standard values have not been finally established but those more generally accepted at present for serum or plasma are as follows The normal concentrations range above 0 7 mg per 100 cc with concentrations of 1 2 or better common in well nourished subjects From 0 4 to 0 7 mg per cent con stitutes a border zone or perhaps one of hypovitaminosis Below 0 4 or 0 3 mg is the level of symptomatic scurvy * These values are, of course, subject to interpretation In par ticular not all subjects with concentrations of 0 4 mg per cent or less will show physical signs of scurvy Many in fact will not Nevertheless, there is good reason to believe that such levels represent a definitely deficient state at which physical signs of scurvy may appear, and may be found on close search if they are not easily apparent Values up to 0 7 mg per cent are probably indicative of at least mild hypovitaminosis Individual exceptions due to unknown causes will of course, occur and all results should be inter preted critically Infants have essentially the same standards as adults indicating higher requirements per unit of weight, and also that weight alone cannot serve as an index of need

The concentration of ascorbic acid in the leukocytes is much higher than in the plasma or serum and with defi ciency does not fall until long after the plasma or serum concentration Hence it is a better quantitative measure of the deficiency and reflects more accurately the scurvy level

Saturation or Load Test This test consists of administer

* Under some conditions there may be a zero concentration of ascorbic acid in the blood for weeks without definite symptoms of scurvy

ing a large dose of vitamin C and observing the effect on the excretion of the vitamin in the urine or its concentration in the blood * In normal subjects the blood concentration rises sharply and a considerable portion of such a dose is excreted in the urine within twenty four hours In the deficient subject all or nearly all of the dose is retained in the tissues and little or none appears in the urine Similarly, the administered vitamin is rapidly removed from the blood stream so that the blood concentration rises more slowly and to a less extent than in normal subjects The test not only detects deficiency but is a quantitative measure of deficiencies in that the amount of vitamin required to raise the excretion in the urine or the concentration in the blood is a measure of the deficiency This can be done either by varying the size of a single dose or by repeating the same dose as many times as is necessary

Many variations of this test have been proposed and used, measuring either the excretion in the urine, the blood concentration, or both The principal variations have been in the size of the dose, the number of doses, the method of administration (by mouth, intramuscularly, or intravenously) and in the time interval during which excretion or blood levels are observed after administration Different methods possess different advantages and drawbacks Those observing concentration in the blood may require repeated venipuncture, although micro methods permit the use of small amounts of blood obtained by 'sticking' the ear or finger Twenty four hour excretion tests are time consuming and require the collection of twenty four hour specimens of urine, with incorrect results if specimens are missed There is also the problem of storage of the urine under conditions which prevent loss of the vitamin No single method has been widely enough used to receive general adoption and many methods have been so little used that there is insuffi

* The technic for determining vitamin C in the blood and urine as used in the load test is given in the Appendix

cient experience for the interpretation of their results The situation is further complicated by the fact that even in very similar tests the doses or other features have been varied sufficiently to make direct comparison difficult if not im possible

Perhaps the saturation or load test which has had the greatest use and has the best established standards is the measure of excretion following the administration of an oral test dose Following a determination of the twenty four hour excretion the subject is given 400 to 800 mg of vitamin C usually in the form of orange juice The orange juice should be titrated so that the proper amount is given Orange juice fortified with pure vitamin C or solutions of vitamin C may be used if care is taken to prevent loss by oxidation before it is administered (by using immediately) The dose is best given after a meal and the diet should obviously con tain little or no vitamin C or the amount in the diet should be computed and reckoned as part of the test dose The test dose for a child is 200 to 400 mg Urine is collected for twenty four hours and the excretion of vitamin C deter mined Although there is no general agreement on standards, the normal individual may be expected to excrete at least 20 to 30 per cent of the test dose If an insignificant amount of the test dose is excreted in the urine, the test may be re peated as often as necessary to attain a normal response The number of test doses necessary serves as a quantitative meas ure of the degree of deficiency Various objections to the test are apparent and some have been described It is un suitable for small children or for those from whom twenty four hour urine specimens cannot be obtained Under these and other circumstances other tests may be used For the details of these tests the reader is referred to the appendix

Capillary Resistance Test This test was devised and used before the chemical tests for vitamin C were available It is based on the well known clinical observation that petechial hemorrhages, presumably due to increased capillary fragility,

occur in scurvy and may be produced in even prescorbutic (but deficient) states by putting additional strain on the capillaries as with a tourniquet or with suction on the skin The test is, of course, not specific and other conditions causing a positive test must be excluded before the presence of vitamin C deficiency can be inferred However, other diseases which cause capillary bleeding are usually easily detected Other irregularities are encountered such as variations in different skin areas Close correlation among the capillary resistance test, blood ascorbic acid concentration, urinary excretion, and physical signs of scurvy is often lacking This is particularly true when tests are made after treatment has been begun However, by standardizing the procedures, tests giving fairly comparable results have been devised the test may prove helpful when other methods are unavailable and when used in connection with other tests

Several tests have been devised The original, like the Rumpel Leede test, consisted of simply placing a sphygmomanometer cuff about the arm and inflating it until the systolic pressure was nearly exceeded, maintaining the pressure for three minutes and observing the occurrence of petechiae Negative (cupping) or positive pressure may be used and the procedure has been standardized in respect to pressure, time, area, et cetera Two methods commonly used are those of Gothlin and of Dalldorf, using positive and negative pressure respectively A detailed description of these two methods is given in the appendix

X-ray In addition to these tests the x ray may be considered as a laboratory procedure It is useful in detecting the extent and severity of the skeletal (and sometimes soft tissue) lesions of scurvy in infants, and may sometimes detect a somewhat atypical case which has been missed on examination It also serves to confirm the diagnosis of mild scurvy in infants or children in whom the diagnosis may have been doubtful on the basis of physical signs and history alone It is of no

value in early or mild deficiencies (hypovitaminosis) and of relatively little value in adults

None of the x ray signs is specific but taken as a whole and considered in the light of other findings they are usually diagnostic when present The difficulty in the early cases is to decide when they are present since they may be simulated by other disease or artefacts They may be listed as follows (a) Deformity of the soft parts from edema or hemorrhage The hemorrhages may be subcutaneous muscular or subperiosteal (b) The so called ground glass atrophy, especially at the end of the shaft with indistinct or lost trabecular markings This resembles simple atrophy or osteoporosis, (c) Cortical atrophy with an apparent increase in the width of the marrow (d) Increased density and widening of the zone of preparatory calcification the white line of Frankel at the epiphyseal ends of the long bones, (e) Similar changes in corresponding areas in the epiphyseal centers of ossification, (f) Zone of rarefaction (Gerustmark in the shaft next to the zone of provisional calcification, (g) Similar changes in the interior of centers of ossification with *loss of trabecular markings* Simple atrophy leaves these markings (h) So called epiphyseal separation, often displaced toward shaft, lateralward, et cetera Separation occurs through the zone of rarefaction, (i) The presence of lateral spurs near the epiphyseal junction resulting from the displacement of the epiphysis or calcification of the periosteum (j) Calcification (ossification) of the elevated periosteum This is a sign of healing (k) Subperiosteal comminuted fractures of the cortex

The gingival manifestations have recently been described in detail by Kruse who includes microscopic changes seen with the slit lamp Acute and chronic lesions as well as various grades of severity in each category are described in a definite sequence of appearance Acute changes may be superimposed on chronic lesions and different parts show

different stages Early in the acute stage the subsurface vascular papillae are engorged and dilated and microscopically the capillaries are enlarged and congested Interdental papillae are first involved, and then the marginal gingiva Later the gum itself becomes red, often with subsurface capillary papillae which are less discrete, showing as bright points against a diffusely red background, giving a mottled appearance Still later the gum is swollen, often glossy, receded, and the subsurface capillaries may not be seen In the chronic process much the same changes occur but edema and infiltration are greater, the gum may be pale, the capillaries marked, and atrophy develops characterized first by pitting best seen with the microscope, later by loss of interdental papillae and marked recession More severe changes, of course, occur in fully developed scurvy Whether these slight and microscopic changes are sufficiently specific to warrant the diagnosis of early or mild ascorbic acid deficiency is not certain The exceedingly slow response to specific treatment in some cases and blood ascorbic acid concentrations at usually accepted normal levels in some cases with these changes argue against such a possibility

TREATMENT

Scurvy and hypovitaminosis C respond quickly, often dramatically, to the administration of vitamin C provided the dose is adequate and absorption satisfactory Even small and inadequate doses may cause considerable improvement but sufficiently large amounts should be given to cause a prompt and complete restoration to normal and provide an adequate store Histologic evidence of repair of the lesions has been observed in a few hours in experimental animals and clinical improvement may be noted almost as quickly The response is observed irrespective of whether vitamin C is given in natural foods or in the pure chemical form Failure of all of the symptoms and signs to improve with equal rapidity need not be taken as valid evidence that the

syndrome of scurvy is due in part to other factors, or that the pure or synthetic vitamin is unable to completely cure scurvy It is a well known fact that single deficiencies are rare in humans and there is the possibility that other deficiencies may be responsible for some of the manifestations in any individual case of scurvy

Fortunately vitamin C is a vitamin which can be adequately supplied by natural foods not only for prevention and protection but in most cases of curative treatment Pure vitamin C, ascorbic acid is available in crystals tablets, ampules Doses and requirements are expressed in milligrams

Prevention The optimum human requirements of vitamin C are unknown and it is difficult to determine with certainty what constitutes a good intake Part of this difficulty is due to the fact that most studies to determine requirements have used as an index of adequacy the amount necessary to prevent the appearance of signs and symptoms of scurvy Clearly this is only a measure of the amount necessary to prevent gross disease and not of the amount needed to maintain good health Recently studies based on newer biochemical tests to determine amounts needed to maintain a normal body store have indicated amounts considerably larger than those suggested in the past On the basis of these studies a good intake corresponding to the recommended allowances of the Food and Nutrition Board would be for infants 30 to 35 mg daily, for children, 50 to 100, and for adults 70 to 100 mg Expressed on the basis of weight these are in the range of 4 to 10 mg per kg (2 2 lbs) for infants, and 1 0 to 1 6 mg per kg for adults with children between Smaller amounts than these will unquestionably protect against scurvy under ordinary conditions and might be considered adequate but mere protection against scurvy does not insure adequate body stores Children require about as much as adults on the basis of weight Requirements seem to depend not only on weight but also on metabolic activity where growth plays a large part

The relatively small store of vitamin C maintained by the body even under good conditions, the relatively narrow margin between health and pathologic changes, the probable beneficial effects of a liberal (optimal) intake and store, and the evidence of a considerable incidence of hypovitaminosis C—or at least a suboptimal intake—in the general population, all combine to emphasize the importance of prevention by an adequate dietary

Foods rich in vitamin C are the citrus fruits (lemons, limes, oranges, and grapefruit), tomatoes, pineapples, currants, raspberries, and strawberries, green vegetables such as peas, beans, lettuce, asparagus, broccoli, brussel sprouts, young cabbage, cauliflower, chard, collards, kale, parsley, kohlrabi, peppers, squash, turnips, and greens of various kinds Milk is only a fair source of vitamin C The vitamin C content of various foods in milligrams of ascorbic acid can be obtained from food tables

The content of the vitamin in the raw foods is not the only factor to be considered, however The virtue of the citrus fruits and tomatoes lies not only in their naturally high content but in part in the fact that their natural acidity preserves the vitamin against destruction Storage, processing preparation (cooking), the amounts consumed, and the variability of different samples are factors of importance Storage and handling have relatively little effect on such fruits and vegetables as oranges and tomatoes if the protective rind and the cell membranes are not ruptured But storage, bruising, and handling of many of the vegetables and fruits reduce the vitamin C content greatly Modern cold storage and canning if properly performed affect the vitamin C content relatively little Allowing canned food to stand after opening may result in considerable destruction of the vitamin content, even when kept at icebox temperature This is particularly true if the natural acidity of the vegetable or fruit is low Properly pasteurized milk retains a fair proportion of its vitamin, but by the time it is con

sumed the value is usually very low Fresh or canned vegetables can be cooked so as to preserve a considerable part of their vitamin C content but too often they are not Long cooking (stewing or boiling), exposure to air (no cover), and the use of soda increase the destruction of the vitamin

What might be termed luxus consumption for an adult (100 mg a day) can be obtained from 200 cc (approximately 6 oz) of a good quality orange juice a day Similar amounts can probably be gotten from twice as much tomato juice, or a corresponding amount of other fruit, plus a liberal helping of properly cooked green vegetables twice a day and help from other minor sources It must be remembered, however, that such amounts, while sufficient for those in health with an initial good store, may not be adequate to protect those whose need is increased by disease, particularly if their store is low in the beginning

Protection Special protection should be provided in the case of those predisposed to develop the deficiency such as infants on artificial feeding nurslings whose mothers diets may be inadequate, those ill with infections illnesses associated with fever or with heightened metabolism, patients on restricted diets, and those whose intake or absorption of food is impaired Patients expected to undergo surgery, especially those with gastrointestinal disease such as peptic ulcer gallbladder disease et cetera, should be mentioned particularly Nursing mothers even those not needing it for themselves should receive supplements for the sake of the infant In fact it would be well if all infants received a supplement early A tendency in the past to give supplements to infants only under special circumstances (prematurity artificial feeding et cetera) was based in part on the false assumption that the relatively small amounts of C sufficient to prevent scurvy were adequate In all these cases, in addition to the general diet it is well to prescribe a supplement, in most cases a specific amount of some of the foods rich in vitamin C, seldom pure vitamin C

Curative Treatment In the milder cases a diet containing a liberal added amount of vitamin C containing foods is sufficient provided there are no difficulties in absorption and the food actually eaten The latter is of considerable practical importance in patients of the lower income group, in children, in the presence of anorexia or food sensitization, or psychoneuroses and other conditions which cause patients to refrain from partaking of a prescribed diet In such cases, known or suspected, it may be advisable to supply the vitamin in the form of ascorbic acid

Considering 50 to 100 mg of vitamin C a good to optimum daily intake in adults, the administration of an additional 100 mg daily should suffice to cure mild or latent scurvy and provide an adequate store This amount can be given by adding 200 cc (6 oz) of good orange or lemon juice, or somewhat larger amounts of other juices such as grapefruit or tomato to the diet The diet itself should contain a liberal supply, 50 to 100 mg for an adult, derived from raw or properly cooked green vegetables and other fruits In all cases the advantage of a 'good diet should be emphasized not only as an aid in recovering from the scorbutic state but in order to educate the patient to the need for maintaining an adequate intake in the future It must be remembered that the development of scurvy or a latent (subclinical) scurvy is of itself an indication that the patient for some reason has a disposition to partake of an inadequate diet, an error which may have a tendency to recur

For the more severe cases of scurvy, for patients who cannot or will not take an adequate amount in the food or for those whose absorption is impaired, the vitamin may be given in the pure form (ascorbic [cevitamic] acid) It is available in tablets or crystals and may be given by mouth, intramuscularly, or by vein For ordinary purposes tablets administered by mouth will be satisfactory

For intramuscular or intravenous injection the crystals are dissolved in sterile water or physiologic salt solution A

ten per cent solution is satisfactory The ascorbic acid should be neutralized with one half its weight of sodium bicarbonate for intramuscular use or a slough may follow the injection due to the acidity of the solution The solution need not be neutralized for intravenous use if doses of only a few hundred milligrams are given well diluted and slowly Larger doses should be neutralized When solutions of the vitamin are prepared they should be used at once because the vitamin is destroyed rapidly under these conditions

Doses to be used when pure preparations of the vitamin are employed vary with the particular needs and objects in different cases In deficient states the body retains the vitamin almost completely little or none being lost by the usual channel of excretion the kidneys The amount of retention parallels the degree of deficiency, a fact made use of in diagnosing the severity of the disease Therefore, it is possible to administer large doses with almost complete retention and if desired a curative amount can be given in one dose However if very large doses are given intravenously the blood level may be raised temporarily so high that some of the vitamin is needlessly lost, an uneconomic procedure Under ordinary conditions therefore the vitamin is given by mouth in amounts varying from a few hundred to a thousand or more milligrams a day It can be added to the milk for children Intravenous administration is ordinarily reserved for those cases in which administration or absorption by mouth is impossible or difficult For example when the lesions in the mouth make administration by that route difficult a few doses may be given intravenously until the improvement permits oral administration In such cases it is best to give it in divided doses to avoid waste in the urine Large doses parenterally may be effective when oral administration fails In practice intravenous administration is preferred to intramuscular because the trouble of neutralizing the solution is avoided In some cases as in infants or young children, the intramuscular route is preferable

So far as the writer is aware, vitamin C has no toxic effect even in enormous dosage Several grams have been given orally and parenterally by the writer and others, and plasma concentrations twenty times normal have been obtained without any ill effects With an adequate store any excess is excreted in the urine Reference has already been made to the possibility of nonspecific reactions from the parenteral administration of unneutralized solutions Occasionally there may be a sensitivity to common foods rich in vitamin C, and perhaps an idiosyncrasy to ascorbic acid

Ordinarily all the manifestations of scurvy respond to an adequate supply of the vitamin and no other treatment is needed Even severe lesions in the mouth and ulcers from periosteal hemorrhages respond to this measure alone, but if one desires a mild astringent mouth wash may be used No local treatment is better than strong applications such as solutions of phenol or silver nitrate Fractures are treated in the usual manner and respond well provided the underlying cause for the fracture is recognized and the proper treatment for the deficiency is given Occasionally hemarthroses cause a deformity requiring corrective surgery later

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7

Vitamin-D and Calcium Deficiencies

Vitamin-D Deficiency

(Rickets, Tetany, etc)

HISTORY

THE MOST characteristic and best known result of vitamin D deficiency is rickets A deficiency of vitamin D causes a dis turbance in the metabolism of calcium and phosphorous In *children* this causes rickets a disease principally affecting the bones during their period of growth and development In *adults* the bones have ceased to grow, the lesions differ from those in children, and the resulting disease is osteomalacia and not rickets In older children in whom bone growth has decreased both reactions occur In the past the confusing interaction of the various factors controlling calcium and phosphorus metabolism has obscured the essentially simple relation between vitamin D deficiency and disturbed cal cium and phosphorus metabolism rickets and osteomalacia The result has been that rickets, and osteomalacia especially, have been confused with other diseases due to a disturbed metabolism of these minerals They have also been confused with other vitamin deficiencies and non nutritional diseases affecting the bones

This is not to imply that all cases presenting softening or demineralization of bone, and the symptoms seen in osteo-

malacia are simple cases of vitamin D deficiency, nor that cases of pure vitamin D deficiency in adults are common since quite the opposite is true So many factors are concerned in the metabolism of calcium and phosphorus that other diseases and other deficiencies may produce disturbances in bone resembling to a greater or less extent those due to vitamin D deficiency, or may have considerable effect on the clinical expression of vitamin D deficiency This is particularly true in the case of calcium deficiency which is probably present to a greater or less extent in many cases of osteomalacia in adults, the clinical condition actually being an expression of the combined deficiency

Nevertheless for the purpose of emphasizing the etiologic role of vitamin D and to clarify the confused attitude concerning osteomalacia, it will be assumed that osteomalacia is an expression of vitamin D deficiency in the adult and is the counterpart of rickets in childhood

Rickets was first described by Glisson in 1650 but rachitic changes in bone have been found in skeletons of prehistoric peoples Interestingly the disease seems to have increased in frequency in comparatively recent times in spite of, or perhaps because of, general improvement in the nutrition and economic status of populations Improved food supplies and better clothing and shelter, while beneficial in certain respects, probably have increased the demand for vitamin D and rendered the supply relatively less adequate, and, with concomitant changes in the type of diet, has favored the occurrence of the disease Increasing urbanization, over crowding, and the rearing of children in the restricted confines of cities with decreased exposure to sunlight have further contributed to the increase in the incidence of the disease in recent times These have been accessory factors only, however and the primary cause is an insufficiency of vitamin D The discovery of vitamin D and its etiologic relation to rickets has been known for only some twenty years

NATURE AND FUNCTION

Vitamin D is not a single substance, some ten or eleven distinct but closely related compounds being known to have antirachitic effects in varying degrees * For convenience the singular term is applied to the group and for practical purposes only two compounds are considered important in medicine at the present

The situation with respect to the nature and relation of the many vitamin D substances is confusing to one without special training in chemistry and the chemistry of the sterols, to which vitamin D belongs, is a special and complicated field even for chemists When the antirachitic activity of irradiated ergosterol was discovered by Hess and Windaus in 1927 it was assumed that this was the one and only true vitamin D and ergosterol the only provitamin D substance It was soon found however, that other sterols were capable of exerting antirachitic activity after irradiation Confusion has arisen because of the number of such substances and their exceedingly close similarity and intimate relationship Distinction between them often depends on mere differences in internal structure The difficulty has been increased by a confused nomenclature special names being used for some products, descriptive chemical terms for others and a host of trade names for commercial products, often several different names are given to one and the same product Nevertheless a knowledge of certain aspects of the chemistry of vitamin D is required for an understanding of its nature and place in clinical medicine and for this reason it is necessary to present a brief discussion of its chemical structure, properties, sources and method of preparation

Vitamin D substances are activated sterols Sterols are

* It has been suggested that related substances which possess some vitamin activity be known as vitamers analogous to isomers

complex substances of large molecular weight and complicated structure often closely associated with, but not related to, fats in plants and animals Cholesterol is a familiar example Certain of these sterols when exposed to ultraviolet radiation undergo a change in their structure with the formation of new substances which are antirachitic Such sterols are said to be *activated* and the new substances formed constitute vitamin D The sterol from which each vitamin D substance is formed by irradiation is known as the provitamin Each separate vitamin D substance has its own individual provitamin and each provitamin yields only its own vitamin D when activated All of these substances, provitamins and vitamins, are so closely related that many show no ordinary chemical differences, even in the usual structural formula, a special three dimensional formula being necessary to show the differences in internal arrangement

Of the several forms of vitamin D the two which are most important in clinical medicine are activated ergosterol and activated 7 dehydro cholesterol Their deviation from the corresponding provitamin is shown below* together with that for a third much less important form, given to show the general relationship of the various forms of vitamin D

Activated ergosterol is known as **viosterol** and the principal active agent when purified and crystallized is known as **calciferol** When ergosterol is irradiated a whole series of substances is formed in a more or less step like process The principal one is calciferol and under proper conditions of

* Provitamin		Vitamin
Ergosterol	+	Activated ergosterol (*viosterol-calciferol*)
7 dehydro cholesterol	irradiation	Activated 7 dehydro cholesterol
22 dihydro ergosterol		22 dihydro calciferol

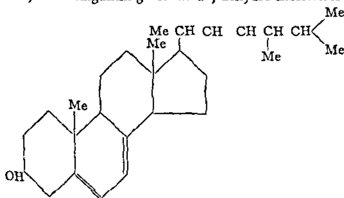
Viosterol is the term applied to irradiated (activated) ergosterol a somewhat impure product Purified and crystallized it is known as *calciferol* The close relation of cholesterol to ergosterol and of activated 7 dehydro-cholesterol to calciferol is indicated by the fact that 7 dehydro cholesterol may be called chemically demethyl dihydro ergosterol and the activated form demethyl dihydro calciferol

activation (manufacture) it constitutes the bulk of the end products Calciferol is also known as vitamin D.

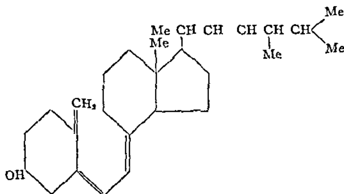
Ergosterol,* the provitamin of viosterol and calciferol, is found chiefly in fungi and yeasts and in some plants It is not a constituent of animal tissues (except incidentally in a few species), cannot be absorbed as such, and hence is not

* The formulas for ergosterol calciferol cholesterol and activated 7 de hydro cholesterol are shown in the accompanying figures Their very close similarity is easily apparent Calciferol has been isolated and synthesized in pure crystalline form Activated 7 dehydro cholesterol has been obtained in pure crystalline form from irradiated synthetic 7 dehydro cholesterol but not from natural sources However crystalline esters of activated 7 de hydro cholesterol have been obtained from fish liver oils

These substances are relatively insoluble in water sparingly soluble in oils and freely soluble in most organic solvents They possess closely similar absorption bands in the spectrum a fact which was responsible for the delay in distinguishing activated 7 dehydro cholesterol from calciferol

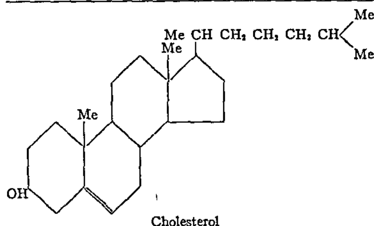


Ergosterol

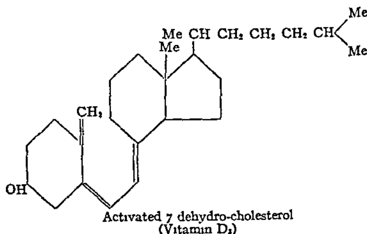


Calciferol

the sterol or provitamin which is activated by sunlight in the skin and hair of animals Irradiated ergosterol (viosterol and calciferol) may, therefore, be considered the plant vitamin D When, however, ergosterol is activated to form viosterol and calciferol it is absorbed and exerts an antirachitic effect Viosterol or calciferol is ordinarily prepared by irradiating yeast or molds When cows are fed irradiated yeast, this is the form of vitamin D excreted in the milk This form of vitamin D milk is called 'metabolized' vitamin D milk and the vitamin D is the plant type, similar to the vitamin D in viosterol in oil Therefore, as will be shown below, it differs from vitamin D milk produced by irradiating milk directly or by irradiating the cow



Cholesterol



Activated 7 dehydro-cholesterol
(Vitamin D_3)

Activated 7 dehydro cholesterol is formed from 7 dehydro cholesterol, a sterol in animal fat It is the vitamin D formed by the action of sunlight (ultraviolet light) on the cholesterol in the skin hair, and feathers of animals and birds It can, therefore be classed as the animal vitamin D or, for them perhaps, the natural vitamin D It appears to be the chief form of the vitamin in fish oils, though fish oils contain other forms of vitamin D and species of fish vary in the relative amounts of the different types of vitamin D in their oils Because fish oil concentrates are widely used in poultry feeds and to fortify milk, this is the form of vitamin D present in eggs and certain vitamin D milks The vitamin D so added supplements the natural vitamin D of the hen and cow

The vitamin D of activated ergosterol (calciferol) and 7 dehydro cholesterol differ not only in their source and in timate chemical structure but also in their biologic activity Activated 7 dehydro cholesterol is equally as effective as calciferol, unit for unit against rickets in rats but is many times more effective in chickens There is some evidence of a similar difference in man

Neither plants nor animals are able to synthesize vitamin D, the vitamin being formed in plants by irradiation of the provitamin after death of the plant and in animals by the action of sunlight on the provitamin (7 dehydro cholesterol) in the skin, hair, and feathers Pre formed vitamin is readily absorbed by animals

As indicated above other vitamin D substances are of little practical importance at present A possible exception is 22 dihydro-calciferol, formed from 22 dihydro cholesterol which may be the sterol of certain plants and may form the vitamin D of irradiated cereals and breakfast foods

The exact site and mode of action of vitamin D is un known There is little doubt that it acts in the intestine and increases the absorption of calcium and phosphorus but

exactly how this is accomplished is not clear An important question is whether vitamin D is active elsewhere in the body As will be seen the concentration of serum phos phatase activity is affected in rickets, being elevated during the active stage and remaining high long after the process of repair has begun Phosphatase activity is present not only in the blood but in the bones the intestine, and other places as well It is possible that vitamin D may be generally concerned with phosphatase activity and active elsewhere than in the intestine to promote absorption of calcium and phosphorus

Vitamin D is also related to the action of the parathyroids When the parathyroids are removed vitamin D is less effec tive though not all of its action is lost In hyperparathyroid ism or when parathormone is administered, more vitamin D is required Hypertrophy of the parathyroids occurs in rickets The exact relationship between them and vitamin D is not known Similarly the thyroid is involved in rickets and lowered basal metabolic rates are encountered in the disease Treatment with vitamin D is followed by a return of the basal metabolic rate to normal These relationships between rickets vitamin D, and other organs and functions suggest that vitamin D exerts a more general effect in the body than a simple local intestinal action on the absorption of calcium and phosphorus

Man obtains the major portion of his vitamin D through the action of ultraviolet light on the sterols (mainly choles terol) in the skin The wave lengths of ultraviolet light which are effective are sharply defined, and this is impor tant because of the effect of various factors in filtering out these particular wave lengths In general the effective range is from 313 to 230 millimicrons, but in sunlight only wave lengths from 313 to 296 are present, a very narrow band In winter the atmosphere may filter out those below 306 thus greatly reducing the number of effective waves Arti

ficial sources carbon arc light or mercury vapor quartz lamps emit wave lengths considerably shorter (down to 230 millimicrons) and are somewhat more effective

Relatively small amounts of vitamin D are taken in with the food under ordinary conditions It occurs in significant amounts in only a few foods, namely certain fishes, eggs, and milk It is of course, present in considerable amounts in some foods not commonly eaten such as fish roe and there is a small amount in mammalian liver The amount in eggs depends on the food of the hen and the hen's exposure to sunlight (ultraviolet light) That in milk (butterfat) depends on the amount of vitamin D and sunlight the cow receives With present practices of feeding, hens may receive liberal amounts of vitamin D Irradiating the milk, or the cow herself, or feeding her irradiated yeast may cause the vitamin D content of the milk to be greater than is normally the case The irradiation of certain other foods may contribute to the greater intake In general however, food contributes only a small proportion of the D, particularly in infants

Vitamin D can be stored in the liver and infants can be born with a very considerable reserve if the mothers are given liberal amounts of vitamin D during pregnancy

Vitamin D is toxic in large doses but the amounts are excessive and much greater than those ordinarily employed in the prevention and treatment of rickets Except in the rare case of resistant rickets a toxic effect is apt to occur only when the vitamin is used in *enormous* doses as in the treatment of arthritis and certain other diseases In those cases doses of hundreds of thousands of units have been employed and even then toxic symptoms are not always produced Obviously this use of vitamin D has nothing to do with prevention or correction of a deficiency and employs vitamin D for a pharmacologic effect The toxic manifestations will be discussed under treatment

PATHOLOGY AND PATHOGENESIS

Pathogenesis of Rickets The essential physiologic abnormality in vitamin D deficiency is a diminished absorption of phosphorus and calcium In most cases this is accompanied by a decrease in the phosphorus concentration in the blood The calcium usually remains normal or is only slightly decreased Normally the infant and child absorbs from 30 to 70 per cent of the phosphorus and calcium of the food A considerable part of this is excreted, phosphorus for the most part in the urine, calcium for the most part in the stools, but sufficient is retained to cause a positive balance, i e , the amount excreted is less than that ingested In the blood of both infant and adult the calcium of the blood is maintained at an exceedingly constant level of 9 11 mg per cent Phosphorus varies somewhat and is higher in children than adults (children 5 0 ± 0 5 mg per cent, adult 2 0 to 3 7 mg per cent) In rickets the urinary excretion of phosphorus is greatly diminished, that in the stools greatly increased The calcium excretion in the urine, normally small in amount, is decreased and the excretion in the stools greatly increased This suggests that absorption of both is much reduced At the same time the phosphorus level in the blood is reduced while calcium is usually little changed Retention of both calcium and phosphorus is much decreased but rarely does this reduced retention proceed to an actual negative *balance or loss (excretion greater than intake)*

In some cases the calcium in the blood is reduced as well as the phosphorus This is usually the case when tetany is present in additi[illegible] lly phosphorus is normal or even [illegible] presc[illegible] ickets but there is some reason to b[illegible] l cases in which some [illegible] has escaped

detection As will be shown below an improper ratio of cal cium and phosphorus is the primary biochemical defect in rickets Theoretically then a low calcium with normal phos phorus might be associated with rickets In fact, however, the common finding in rickets is a low phosphorus, with a normal, or low calcium

Under the influence of vitamin D alone the abnormal absorption and excretion of phosphorus and calcium is cor rected Phosphorus excretion rises in the urine and falls in the stools The calcium excretion in the stools is decreased and the excretion in the urine restored to normal Blood phosphorus rises to normal, as does calcium if it has been lowered A greater than normal retention of calcium and phosphorus occurs, amounting for a time to as much as 60 80 per cent of that ingested Only under conditions of extreme calcium and phosphorus deficiency does vitamin D fail to produce this change Such a state is not only rare but is pri marily a state of phosphorus and calcium deficiency and not vitamin D deficiency

In order to understand the effect of these biochemical disturbances on the absorption, excretion, and blood con centration of calcium and phosphorus it is necessary to know something of the chemical process of lime salt deposi tion in bone Calcium salts are deposited to form true bone by a process of precipitation and adsorption ' Briefly and simply the process consists in the precipitation of calcium phosphate from the blood into the osteoid tissue and the *adsorption* of calcium carbonate (In addition to calcium other such ions such as magnesium, iron fluorine sodium and potassium are absorbed in minute amounts) The result has been described as a solid solution of calcium carbonate in calcium phosphate Calcium phosphate is present in the blood in a supersaturated solution together with calcium ions Some process such as the addition of extra phosphate

ions raises the calcium phosphate concentration to a point where its solubility is exceeded and it is precipitated in the osteoid tissue Calcium carbonate is later added by adsorp tion The precipitation of calcium phosphate occurs in a special localized area only, namely in the zone where active bone formation is taking place The exact mechanism whereby the change in the solubility of calcium phosphate in the blood and its precipitation is brought about is un known There is some reason to believe that local phos phatase enzymes may 'flood the local tissue fluid with extra phosphate ions by splitting organic phosphates and freeing inorganic phosphate ions Proper alkalinity favors this process

In vitamin D deficiency, with a lowered concentration of phosphorus in the blood proper physiochemical conditions (proper concentration and proportion of phosphorus, cal cium, and calcium phosphate ions) for the precipitation of calcium phosphate are lacking, the lime salts fail to be de posited, and the osteoid bone is not calcified Since calcium is concerned in the process an abnormal supply of calcium and an improper ratio of calcium to phosphorus may also affect the process but the common fault is a lowered concen tration of phosphorus Such changes as occur in the bone are apparently the result of this primary biochemical lesion

Although a deficiency of vitamin D with the resulting disturbance in the metabolism of phosphorus and calcium is the essential cause of faulty bone formation in rickets, secondary factors (as is the rule in deficiency diseases), may have an influence on the occurrence of the disease

These secondary factors include (1) the absolute amounts and relative proportions of calcium and phosphorus in the diet, (2) the availability of this dietary phosphorus and calcium, (3) the reaction of the diet, whether acid or alka line, both in the intestine and in the body after absorption, (4) factors other than vitamin D deficiency causing a loss

or increased excretion of phosphorus and calcium and finally, (5) factors influencing the rate of growth, general nutrition, and the metabolic level These factors by increasing or decreasing the need for calcium phosphorus, and vitamin D, may render the supply of these substances adequate or relatively inadequate

It has been found that both the amounts of phosphorus and calcium and the relative proportion of each affect their absorption An excess of calcium, perhaps by carrying out phosphorus with it may make an otherwise adequate intake of phosphorus insufficient An excess of phosphorus may cause likewise a loss of calcium Diets low in both phosphorus and calcium will be more suitable if there is relatively more phosphorus than calcium Conversely diets high in calcium require relatively more phosphorus than otherwise Such diets harmless if there exists a liberal supply of vitamin D, may in the presence of a borderline or minimal supply of vitamin D, serve to tip the balance and render an otherwise adequate vitamin supply insufficient

Diets may contain what appears to be adequate total amounts of phosphorus yet much of it may be unavailable in the form of inorganic phosphorus For example, certain cereals (oatmeal) contain large amounts of phosphorus but much of it is in an organic form not available to the body

Acidity in the intestine favors the absorption of phosphorus and calcium alkalinity decreases it Acidity of the blood and body fluids hinders the deposit of calcium alkalinity favors it Diets with an acid reaction in the intestine and an alkaline ash when burned in the body tend to protect against rickets Diets of the opposite type tend to favor its occurrence Though in itself not a primary cause of rickets in man diet may render a slender supply of vitamin D insufficient An example of the effect of alkalinity and acidity is given by the action of a simple mixture of citric

acid and sodium citrate When ingested its acid reaction in the intestine favors absorption and when burned in the body it leaves an alkaline ash, favoring the deposition of calcium salts * This citrate mixture alone may cure mild rickets Though it has been found possible to produce rickets in rats by modification of diet alone this does not occur in man

Gastrointestinal disease or disorders may lead to a deficient absorption of calcium and phosphorus, usually by increasing the loss in the stool Celiac disease with increased fat excretion leads to large losses of calcium (low calcium rickets) though it is probable that this lost calcium carries out phosphorus with it, a more significant loss High fat diets may have a similar effect

Finally, factors influencing growth and general nutrition have an important influence Starvation or undernutrition may not be accompanied by rickets, and in fact may ' heal rickets if it is already present Good nutrition, favorable in other ways, may by increasing the growth rate require larger amounts of vitamin D than are available Because of this, many other conditions affecting health, such as infections, metabolic disorders, and endocrine disturbances may modify the occurrence and course of the disease Finally, sporadic cases occasionally occur in which the exact reason for the vitamin-D deficiency and the development of rickets cannot be determined

In considering these secondary factors it is important to emphasize that their influence always depends on the existence of the primary defect, a lack of vitamin D In other words, amounts of the vitamin which might be otherwise adequate are rendered insufficient by adverse secondary

* Chemically the reaction is not quite as simple as indicated the citrate forming a complex compound with calcium the exact significance of which is not clear

factors, or, smaller amounts would suffice and the body would be 'protected' if secondary factors were favorable

In adults the pathogenesis as well as the pathology of vitamin D deficiency differs from that in children Pure vitamin D deficiency is very rare and most 'late' rickets occurs in older children in whom some growth is still taking place Bone *growth* has ceased in adults The skeleton constitutes a store house for calcium and phosphorus which can be drawn on as vicissitudes of need and supply require In fact the function of the skeleton as a warehouse with active withdrawals and deposits is of much greater importance than has been appreciated in the past With an inadequate supply of vitamin D calcium absorption is hindered and in order to maintain a normal calcium concentration in the blood which is not decreased except in extreme cases, calcium is withdrawn from the bones

In adults the need and demand for vitamin D is much less than in children and the opportunities for obtaining it, particularly through exposure to sunlight, are usually greater It is probable that the secondary factors already discussed under the pathogenesis of rickets are also operative in adults

The gross changes in the skeleton are almost as easily observed on physical examination as on closer inspection the most characteristic changes being the deformities These as a rule are most apparent and appear first in the long bones, the distal radius ulna tibia, and fibula, and the ribs The flat bones of the head may be affected early, especially when rickets occurs in the first few months of life In general the order of appearance, the severity of the active process and the magnitude of the deformity in different bones vary with the age at the onset of the disease and with the duration and severity of the disease The rate of growth of various portions of the skeleton at different ages and strains of muscle action and posture at these periods greatly

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Microscopic Changes A characteristic feature of the microscopic anatomy of rickets is the disorderliness and dis arrangement of the pattern of bone structure which in health is one of great orderliness and regularity In order to understand the significance and development of this dis array, it is necessary to understand the architecture and pat tern of normal bone growth Since rickets occurs in most cases some months after birth, a time when ossification of a large part of the bone (shaft or diaphysis) has occurred and affects principally the region of the junction of the epiphysis and diaphysis where most active growth and ossification are occurring, a description of the cellular process of ossification in this area is most suitable A brief description of the proc ess of ossification in flat bones and the changes in rickets will be given later

Normally the region of the junction between epiphysis and shaft is divided into three zones The distal zone of the epiphysis is composed of resting cartilage, the cells of which are arranged in a somewhat irregular fashion but with a tendency to a transverse pattern Toward the shaft the sec ond zone is that of proliferating cartilage which is the region of active growth and ossification It is this area which by its growth and ossification adds to the length of the long bones In this zone the cartilage cells are arranged in parallel rows lying in the long axis of the bones These rows of cartilage cells are separated from one another by a jelly like collagenous material containing cells known as osteoblasts These rows of cartilage cells lying in their collagenous ma terial end where the shaft or third zone begins At the junc tion of these rows of cartilage cells and the shaft there is a line or zone of capillary loops which form a smooth, even surface at the end of the shaft from which they originate During the process of bone growth and ossification these capillaries invade the columns of cartilage cells destroying them as they (the capillaries) advance A calcification of the

influence the localization and severity of the lesions All bones, however, may be affected At very young ages (first few months) the skull is apt to show extensive involvement In the ordinary case, mild to moderate in severity and occurring in the latter part of the first month, the distal ends of the radius, ulna, tibia, and fibula are most apt to present significant changes The long bones show thickening of the shaft, are softer than normal, and there is usually some enlargement at the epiphyseal junctions In the ribs the enlargement takes place at the costochondral junction Distortion of contour with bending of the shaft in various planes dependent on weight bearing and muscle pull appear somewhat later In addition to the thickening of the epiphyseal junction the epiphysis may be bent out of line with the shaft The bones of the skull may show areas of thinning of the inner table, so severe in some cases that the bone is reduced to parchment thinness (craniotabes) In other areas particularly over the parietal and frontal bosses there is thickening Fractures, often multiple and usually either of the green stick variety or partial fractures (one side only), are common in the long bones In severe cases there may be arrested skeletal growth On longitudinal section there is a general hyperemia, the junction of shaft and epiphysis is widened, lengthened, and softer than normal The medullary portion is red and soft, the bony trabeculae thinned and the central cavity widened The periosteum is hyperemic and under it the tissue is more vascular and friable The irregular architecture and alteration in the usually clean cut zones is visible, even in the gross During healing many of these changes disappear, but a long time may be required for the return to normal of the finer changes in architecture, and certain deformities, notably those of contour and thickening, may persist as a permanent scar

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Normally the region of the junction between epiphysis and shaft is divided into three zones The distal zone of the epiphysis is composed of resting cartilage the cells of which are arranged in a somewhat irregular fashion but with a tendency to a transverse pattern Toward the shaft the second zone is that of proliferating cartilage which is the region of active growth and ossification It is this area which by its growth and ossification adds to the length of the long bones In this zone the cartilage cells are arranged in parallel rows lying in the long axis of the bones These rows of cartilage cells are separated from one another by a jelly like, collagenous material containing cells known as osteoblasts These rows of cartilage cells lying in their collagenous material end where the shaft or third zone begins At the junction of these rows of cartilage cells and the shaft there is a line or zone of capillary loops which form a smooth even surface at the end of the shaft from which they originate During the process of bone growth and ossification these capillaries invade the columns of cartilage cells destroying them as they (the capillaries) advance A calcification of the

collagenous frame work or wall between the rows of cartilag cells slightly precedes the advance of the capillaries Th calcification appears to be due to the action of osteoblast As the capillaries reach those areas of calcified intercellula substance the latter is decalcified and osteoid tissue i formed by the osteoblasts This forms the organic fram work of the bone and, as the advancing process continue the lime salts of true bone are deposited in this osteoi tissue under the influences of the physiochemical processe already described Thus, behind the advancing zone o ossification true bone appears and lengthens just as the soc grows behind the knitting needles and the yarn that is fe into them As the bone grows in length it grows in thicknes by the laying down of additional bone under the periosteum by much the same process As growth and development con tinue there is at the same time an active resorption of bone particularly in the trabeculae of the cancellous portions This results in a rearrangement of the internal pattern to correspond to lines of stress and strain which develop a weight bearing, muscle pull, and other effects of use and function occur To some extent resorption and production of bone occurs throughout life, particularly in the less com pact areas, as variations in the supply and demand of calcium occur *In this way the skeleton serves as a store* house fo calcium much as the liver stores glycogen

An essential feature of the process of bone growth is its orderly progress and pattern, the capillaries advancing at an even pace, on an even front, the cartilage cells disappear in an orderly line just before them, and the advancing pre liminary calcification of the collagenous frame work is fol lowed by an orderly and even line of osteoid tissue under process of being ossified In the x ray this orderly process is apparent in the sharp, narrow white line of advancing ossification

In rickets all this is changed In the zone of proliferation

the cartilage cells develop abnormally Instead of degenerating evenly and uniformly just before the capillaries reach them, they are resistant to degeneration and fail to be destroyed and absorbed by the advancing capillaries The region of proliferating cartilages enlarges because of the failure of the cells to die and large masses of cartilage cells pile up in irregular fashion Although the capillaries eventually reach the cartilage cells and destroy them the process is very irregular In some places the capillaries succeed in breaking through and develop into large bunches with trunks and many branches In other places they fail, giving a very irregular line of advance and even when the capillaries have penetrated, isolated masses or islands of cartilage persist The cells on the outside of the masses are abnormal in appearance and staining properties due to mechanical distortion and the destructive action of the capillaries Some of the original matrix between the cartilage cells is destroyed but some persists, both calcified and uncalcified On this the osteoblasts accompanying the capillaries lay down osteoid tissue and build irregular chondro osteoid trabeculae, abnormally shaped and running in all directions In this osteoid bone there are inclusions of cartilage cells Depending on the severity and stage of rickets this osteoid bone tissue either completely fails to calcify or does so very incompletely and irregularly

This rachitic intermediate zone, between the generating part of the proliferative cartilage and the shaft, is a disorderly mass of proliferative cartilage bushes of capillaries and osteoid or partially ossified trabeculae running in irregular directions, some at an angle, some close together, some widely separated In the more severe cases vessels really extensions and enlargements of the cartilage canals penetrate this zone from the distal side (resting cartilage side) as well as from the shaft and less often from the side (perichondrial) These vessels are never very large nor do

they penetrate deeply Connective tissue brought in by the blood vessels may be greatly increased

It is this rachitic intermediate zone which accounts in a large measure for the deformities Its enlargement causes enlargement of the ends of the long bones as they increase in length and pushes" the centers of ossification of the epiphysis further away from the shaft Being osteoid (soft) bone it is easily bent by forces of posture, weight bearing, and muscle pull The direction of the line of growth is thus changed and growth continues to follow the new direction even when it is at a considerable angle to the line of the shaft previously formed With its greater size the inter mediate zone becomes too large for the shaft, spreads the shaft, and deflects the trabeculae outward Increased thick ness of the shaft develops from increased perichondrial de posit of osteoid tissue Improper calcification and increased decalcification of the shaft softens the latter which bends into various deformities Bending of the shaft is usually one of the later deformities

Recovery and Repair When vitamin D deficiency ceases repair begins Bone growth and calcification become nor mal as soon as adequate vitamin D is available and the proc ess begins in the region where normal ossification would be occurring had rickets not intervened This is in the distal or cartilage end of the intermediate zone leaving the mass behind to be straightened out later The normal ossifica tion resulting gives rise to the dense transverse white line of *healing* seen on x ray plates Next, focal areas in the mixed mass of tissue in the intermediate zone calcify, be ginning in the area most favorable for calcification Gradu ally the vessels (capillaries) assume a regular order and con vert the osteoid trabeculae into normal bone Abnormal destruction comes to an end but because there has been an excessive irregular overgrowth of osteoid tissue there is a gradual, selective absorption, reducing the mass of tissue,

straightening it out, and rearranging it as far as possible into the normal pattern To complete such a process may take months or years but the degree of restoration is sur prising Internal abnormalities may disappear, but deformi ties due to bending may persist as well as some thickening of the trabeculae

Changes similar to those described at the epiphyseal diaphyseal junction of growing long bones occur in all bone in the process of formation, the osteoid bone everywhere being deficient in calcium salts In ordinary sections the trabeculae when cut in a single plane exhibit borders of non lime containing osteoid tissue Such changes can be seen in the cancellous portions of long bones in the cortex along the lacunae, in the tables of the skull and elsewhere in the skeleton

Defects in tooth structure may be assumed to occur in rickets although the differentiation between injury due to vitamin D and those possibly due to vitamin A and vita min C deficiency (as well as other deficiencies) has not been clearly established in man In vitamin D deficiency the lesions are most common in the permanent teeth because rickets usually develops at the time calcification of this set is occurring However defects in the temporary teeth pre sumably due to rickets do occur and the likelihood of vita min D deficiency as a cause is increased by the similarity to the changes found in cases of congenital rickets

Other pathologic changes are lacking in uncomplicated rickets except a slight hypertrophy of the parathyroid glands and perhaps some atrophy of the thyroid cells The fibrosis and atrophy of muscles seen in some cases are prob ably not specific for rickets

In adults and in the non growing bones of older children the pathologic changes in the bones are those of osteo malacia The pelvis spine, and bones of the extremities are more apt to be affected but any part of the skeleton can be

involved in severe cases Softening, bending, and deformity of the bones occur (for example the osteomalacic pelvis in women) and fractures are more common than in rickets There is thinning and atrophy, demonstrable on x ray examination, and microscopically an excess of osteoid tissue If the deficiency is severe hypocalcemia may develop and tetany ensue

The pathologic changes caused by the toxic action of excessive vitamin D is essentially a general calcinosis With *this there is a hypercalcemia which usually is present as a* warning some time before the actual deposit of calcium occurs

INCIDENCE AND EPIDEMIOLOGY

The exact frequency of rickets is very difficult to determine and varies greatly under the influence of climate, geography urbanization, season of the year, and the practice of preventive treatment At the beginning of this century rickets was almost universal in the temperate zone in western Europe and America The classic postmortem studies of Schmorl revealed an incidence of 94 to 98 per cent among the children, four to eighteen months of age, in Vienna and rickets was undoubtedly the most frequent deficiency disease at that time With the development of our knowledge of the cause and prevention of the disease and the introduction of protective measures, both the frequency and severity of the disease have decreased greatly and incidence in a locality is largely a reflection of prophylactic treatment and an index of the level of infant care in the community Nevertheless, even recent studies indicate that if the x ray and other of the finer methods of diagnosis are used, mild or slight rickets is fairly frequent at the more susceptible age levels reaching perhaps a frequency as high as 25 to 30 per cent at six months of age in some localities

Since ordinarily the body is dependent for the greater part of its vitamin D on the action of the ultraviolet rays of sunlight on the skin and hair, the factors controlling ultra violet radiation and exposure to the radiation are of great importance in determining the incidence of vitamin D de ficiency and rickets Ultraviolet radiation is greatest near the equator and decreases as it moves toward the poles Be cause of the movements of the sun and earth the amount of ultraviolet radiation varies with the season (except in the equatorial zone) being greater in summer and less in winter, reaching the maximum and minimum at summer and winter solstices respectively Also it is filtered out by the atmos phere and with increasing latitude the amount of ultra violet radiation is not only less but is of shorter duration There are variations during the day and the periods of effective radiation are much longer in summer (more hours per day) and very short in the winter For example it has been calculated that in the temperate latitude exposure dur ing the whole day in winter would be required to be effec tive and that in many parts of that zone no effective radia tion occurs after 3 00 p m in winter

The ultraviolet waves are blocked by smoke and dust and by clouds Fortunately it is not necessary to be exposed to direct sunlight to receive ultraviolet radiation and skyshine is in many respects as valuable or more valuable than direct sunlight It begins earlier in the day and lasts longer, may be quite strong with light cloudiness and in the winter it is actually more valuable than direct light To obtain the maximum amount of skyshine, however, there must be no obstruction of the horizon, and walls, buildings, et cetera, block a large part of it

Ultraviolet radiation is blocked by practically all cloth ing and by ordinary window glass Hence colder weather of the higher latitudes, by increasing the need for clothing and shelter, tends to decrease the amount of ultraviolet radiation

received The radiations are less well absorbed in dark skinned persons, negroes particularly

The effect of these factors on the incidence of vitamin D deficiency and rickets is apparent Their effect is more marked in the northern latitudes, and rickets is essentially a disease of the temperate zone (the diet of the Eskimo protects him unless contact with civilization changes his natural diet) Incidence is greater in the crowded, smoky cities, and much greater in winter and spring than in summer or autumn Children who are born so that their most susceptible period (four to six months) comes at a time of relatively greater ultraviolet radiation are less likely to develop a deficiency of vitamin D and rickets A deficiency is more apt to occur among those confined indoors and in dark skinned races This is easily shown by the greater incidence in negroes, particularly among those living in a less favorable climate (northern cities) It must be remembered, however, that rickets is due to a deficiency of vitamin D and if a deficiency of the vitamin occurs, rickets may be encountered in any region

Congenital rickets is rare but does occur Rickets is very common and apt to be severe in premature infants and twins The period of maximum susceptibility and incidence is in the latter months of the first year It is uncommon before two months which is about the length of time it takes rickets to appear clinically It becomes progressively less frequent after the first year of life and is quite infrequent during puberty

Little is known regarding the incidence of vitamin D deficiency in adults As osteomalacia it is recognized to be more common in women during the child bearing period, associated with pregnancy and lactation It also is related to poverty, famine, and overcrowding However, osteomalacia no means of estimating the incidence of mild deficiency as such is an expression of a severe deficiency and there is

Furthermore, the deficiency is closely associated with a deficiency of calcium and the two deficiencies are often combined to produce the clinical disease Because of the greater opportunity of exposure of the adult to sunlight, it is unlikely that pure vitamin D deficiency is common However, large numbers of adults and many older children have little or no exposure to sunlight due to their occupations, and many certainly have little or no vitamin D in their diet Hence associated with mild calcium deficiency, vitamin D deficiency may be fairly prevalent among certain classes of individuals (See section on Calcium Deficiency)

SYMPTOMS AND SIGNS

It is clear that the signs and symptoms of the disease will vary according to many factors Perhaps the primary factor is the degree (severity) of the deficiency but this is relative and is greatly modified by other factors such as the age of the patient the *rate* of growth and development, and the location of this growth and development at the time of deficiency Poor nutrition generally by affecting growth, may modify or inhibit rickets Starvation cures rickets Mild and severe rickets differ greatly in the very young Deformities depend a great deal on the stress and strain placed on the various bones and these in turn differ according to their stage of growth and development It also follows that the duration of the deficiency modifies the clinical picture

In general the symptoms of the disease are mild and non specific and the principal evidence of the disease is the visible and palpable alterations and abnormalities of the skeleton The onset of rickets is usually insidious and skeletal changes are often the first thing observed In young infants these are first evident at about three months as craniotabes In the usual case this is observed in a few small areas on the back of the head where at this age pressure is

relatively great and constant The sutures are widened and the edges soft Somewhat later beading of the costochondral junctions (rachitic rosary) appears and an enlargement of the wrists (lower radius and ulna) may be noted If the dis ease begins somewhat later craniotabes may be absent Con stitutional symptoms are generally lacking and the infant appears well and gains weight With more severe rickets, however, restlessness, head sweating, irritability, and weak ness may be observed and the clinical picture commonly seen at a later age may make an earlier appearance

During the middle part of the first year extensive cranio tabes, obvious malnutrition, and weakness occur more fre quently and are more pronounced Cranial deformities ap pear due to the flattening of the back or the side of the head A little later (eight or nine months) craniotabes disappears but the cranial deformities increase, and frontal and parietal bosses or prominences begin to give the ' square ' head and ' Olympian brow ' The anterior fontanelle is wide and the posterior remains open The rachitic rosary is well marked during the sixth to eighth months and is succeeded by the other deformities of the chest such as Harrison s groove, depression of the sternum, lateral trough and prominence of ribs parallel to the sternal depression There is increasing enlargement of the distal ends of radius and ulna, although this may be obscured by fat While the infant may be fat and may *appear* well nourished, the tissues (the musculature especially) are flabby, the color is often pale, and weakness is usually evident Activities normally present may be lack ing The abdomen is frequently prominent (pot belly) but this may not appear because of the undernutrition

If rickets continues in a moderate or severe form during the first and second years these signs, except for craniotabes, persist or increase If the onset of rickets occurs at this time, these signs become quite pronounced Muscular strength and development are poor, and as a result the child fails

to stand and walk The deformity of the head becomes more apparent (caput quadratus or hot cross bun head) and the rachitic bosses are more prominent Harrison s groove and other deformities of the chest increase, the spine may become bowed (kyphosis) and deformities of the leg (tibia and femur) appear such as bowing and enlargement of ankles and knees The fingers may show enlargement of the proximal and distal phalanges with constrictions at the joints, and the pot belly may increase Dentition is delayed, and defects in the enamel and caries appear Movement and handling may cause pain and x rays may reveal one or more green stick fractures of the long bones The anterior fon tanelle may be open at the age of two or older At two or three years the child may not be able to stand or walk When raised to a sitting position rachitic children assume a typical attitude of support with the legs crossed and the hands resting on the floor by their sides for support If walk ing is acquired the deformities of legs and pelvis may be increased and the walking itself is clumsy and poorly co ordinated Angular deformities at the epiphysis occur at this age (two to three years) while deformity of shaft is seen more frequently after four years of age

Certain of the deformities require further comment and description In general they are the product of the severity of the deficiency, the developmental stage of the part af fected and the stress and strain to which that part is sub jected Thus in the case of the infant lying on its back the head, the chest and pelvis bear the greatest weight As the child sits up the spine is under a special strain and the pelvis is subject to a different pressure Standing and walk ing add new stresses and strains At the same time the de formity will depend greatly on the severity of the rickets The more rapid the growth of the part the more susceptible it is to rickets, and at the same time the rapid growth in creases the severity of rickets

Craniotabes is a descriptive term and not necessarily a lesion of rickets It consists of a softening and thinning of an area in the bones of the skull This area indents and re bounds often with a crackle like a thin metal plate Similar changes are found in osteogenesis imperfecta, in hydro cephalus and even in some normal (especially premature) infants The condition is due essentially to a lack of calcifi cation of the bone In rickets the areas are spotty, nearly always posteriorly located in the parietal and upper occipital area, and have poorly defined edges The size varies from small (2 cm) up to very large areas and they are usually not symmetrical They may appear as early as the third day but are more common at three or four months and disap pear as a rule before the end of the first year Occasionally they persist up to the first year and a half The lesions in osteogenesis imperfecta are usually larger and more widely distributed Similar areas in normal infants are usually uni lateral, are located along the suture lines, disappear before rachitic craniotabes usually occurs, and are not accompanied by other signs of rickets Associated changes in hydro cephalus are usually sufficient for the differential diagnosis The presence of craniotabes is said to be the ' most reliable single, bedside sign of rickets

The bosses' on the skull are located most frequently in the frontal and parietal regions The thickening occurs on the surface because the new bone is mainly of periosteal origin, and is therefore elaborated in the central rather than marginal portions of the bone In the forehead these bosses cause the Olympian brow while parietal bosses give the characteristic "square head of rickets Long narrow heads may, however, have extensive rachitic changes Although thickening gives an appearance of enlargement to the head the cavity is not increased in size and the circumference is usually normal though it may be increased

Closure of the *fontanelles* is delayed and the anterior

fontanelle which normally closes at eighteen to twenty four months may persist as long as three to four years, perhaps actually enlarging as the head grows The margins are soft and difficult to outline After the age of two the persistence of an anterior fontanelle should suggest the presence of cretinism or hydrocephalus though either may occur with rickets Rickets does not cause hydrocephalus

The changes in the thorax are characteristic Commonest is the beading of the costochondral junction (rachitic rosary), but slight beading due to rickets is difficult to distinguish from the slight enlargement which is often normally found Much of the enlargement in rickets occurs on the inner surface where it is difficult to detect, particularly if there be a retraction of the ribs at this point If the rickets is at all severe deformities of the chest occur and persist as the rickets heals leaving permanent evidence of the disease Common deformities are a flattening of the chest, a vertical depression parallel to the sternum from about the third to the ninth rib, with a prominence of the sternum which may be curved or bent giving the pigeon breast In other cases the sternum is depressed In most cases some respiratory obstruction is needed in addition to the rickets to produce an obvious pigeon breast Along the attachment of the diaphragm there is often a shallow depression (Harrison s groove) of the ribs which is exaggerated by the flare of the lower ribs This flare is exaggerated by abdominal distension

In the spine kyphosis develops with the sitting posture, changing to a lordosis (lumbar) when standing and walking are accomplished The deformity is not fixed and can be corrected passively, but often relapses due to lack of muscle tone Pain and spasm are absent unless there be a fracture of the ribs or vertebrae

The worst deformities of the pelvis occur when severe rickets is continued on into childhood and particularly

when the child is sufficiently active that it can stand and walk in spite of the rickets These deformities which are familiar to the obstetrician may be most apparent on x ray and often constitute an important complication of preg nancy

Deformities of the shafts of the long bones are generally a relatively late deformity, though they may occur earlier, associated with sitting, standing, and walking For example, the saber shin usually develops with sitting but can occur with the infant lying Several deformities may occur in one place, for example the legs may show not only lateral but also anterior bowing Such deformities produce knock knees and bow legs As age increases various shifts in the position of the deformities as well as changes in the thick ness of the bone occur Thus, in saber shins the forward bend which in infancy is just above the ankle ascends with growth until it comes to lie at the junction of lower and middle third At the same time there is a thickening of the shaft in the anterior posterior diameter and some thinning laterally In contrast to the saber shin of syphilis, the rachitic thickening is irregular and the bowing is anterior only, not being confined to the lower third of the shin Coxa vara, a bending of the neck of the femur on the shaft to a more acute angle, can cause the waddling gait

In addition to the head sweating irritability, restlessness, and head rolling, head shaking and nystagmus (spasmus nutans) may appear The nystagmus is peculiar in that it may occur in any plane, in either or both of the eyes and, if bilateral, is convergent Its exact relation to rickets is un known

DIAGNOSIS

The diagnosis of rickets is based on the symptoms and physical signs of the disease, the evidence on x ray exam

ination, the determination of phosphate and phosphatase concentration in the blood, and a therapeutic trial Analysis of the diet except with respect to the inclusion of vitamin D preparations is of little value, although the history of a poor diet may suggest a deficiency that would otherwise be unsuspected The need for supplements to infants under ordinary conditions is so frequent that in actual practice the lack of prophylactic treatment is in itself enough to warrant a tentative diagnosis of mild rickets which may be confirmed by x ray or other tests Under such circumstances the patient often exhibits mild symptoms and early physical signs of the disease These have been described but emphasis should be laid on the earliest manifestations Restlessness head sweating, irritability, with or without early changes in the skull, ribs, or long bones should suggest rickets and, if necessary, specific tests should be performed

However, most of the signs are, in the light of our present ideas of the deficiency, rather late signs and are indicative of advanced disease in spite of the fact that advanced rickets as classified by earlier standards is much worse In the truly early or mild cases such signs are either absent or uncertain and dependence must be placed on x ray signs and other laboratory tests

The x ray examination gives clear evidence of rickets in the moderate and severe cases but it fails to give a much earlier diagnosis than a careful history and physical examination Early roentgen changes are apt to be uncertain and inconclusive With x ray however one can follow the lesions at frequent intervals observing the effect of treatment and thus utilizing to greater advantage the therapeutic test

For reasons of availability (the costochondral junctions would be preferable but do not lend themselves well to examination) and convenience, as well as for early development of changes, the lower radius and ulna are the best sites for the clinical x ray examination No more than a

brief description of these x ray signs will be given For further description the reader is referred to more comprehensive treatises on rickets or to books on radiology Interpretation, particularly in the early cases, must be left to the roentgenologist or to a pediatrician trained in the x ray diagnosis of rickets

The principal changes seen in x ray pictures of rachitic bones are cupping, spreading spur formation, fringing stippling, and certain changes in the shaft None of these is specific or pathognomonic, all are subject to the doubt of interpretation Nevertheless, in association with other evidence, they are a useful means for the diagnosis of moderately early rickets, particularly in conjunction with the test of treatment

Cupping is a concavity at the end of the shaft Cupping does not occur in all bones and is not present consistently even in late cases It may occur in scurvy and under some circumstances even in normal subjects Spreading is a widening out of the end of the shaft which may or may not be accompanied by cupping and also is seen in scurvy Cortical spurs are calcified lines extending from the cortex of the shaft along the side of the proliferative cartilage Often they are not in good alignment with the cortex They are found in congenital syphilis and to a slight extent at times in normal bones Fraying or fringing is caused by narrow thread like shadows running from the shaft into the cartilaginous portion Characteristically irregular and tangled in direction, thin and short in the earlier cases and thick and long in the more severe, they are the most reliable x ray evidence of rickets but are not particularly early signs They also occur in congenital syphilis Stippling, which gives an irregularly dotted appearance to the end of the shaft may appear early in the disease but is also seen in advanced cases Certain changes have been noted in the shaft of the bone Eliot[1] describes, in what she terms early in contrast



Fig 13 Bone changes due to rickets There is flaring of the ends of all the bones there are coarse trabeculations and generalized rarefaction of the bone shafts yet the cortices are of normal thickness The cartilaginous changes are particularly prominent at the ends of the radii and ulnae

to very early cases a lack of definition on the edge of the distal end of the ulna away from the radius and a decreased density of the cortex there This is not to be confused with normal irregularities associated with the attachment of the pronator quadratus Similar changes occur in the middle of the shaft of the radius and at the proximal end of the ulna on the radial side as early evidence of periosteal prolifera tion and decreased density of the cortex in these regions Atrophic changes are quite characteristic but are late' manifestations These include decreased density with coarse irregular trabecular markings, a thin cortex hair like shadows (slightly calcified osteoid tissue) extending out from the shaft, a hypertrophic thickened cortex with greater thickening on one side, a narrow marrow cavity, periosteal encasement, et cetera In addition there may be other severe changes such as fractures, distortions, displacements of the epiphysis et cetera

As previously stated the x ray detects changes induced by treatment (healing) It thus gains in diagnostic value and also allows one to follow the progress of treatment Recov ery is marked by the deposit of lime salts This is well seen at the cartilage shaft junction in the form of a transparen line, at first faint and incomplete, and later broad and heavy (sometimes even appearing double) The deposit of lime salts occurs first in the region where calcification would be occurring had rickets not developed, this being the distal portion of the intermediate zone Thus the line is located a considerable distance from the end of the shaft a distance dependent on the chronicity and severity of the rickets Later the intermediate zone shows spotty shadows as the osteoid trabeculae become calcified and lime appears in the periosteal encasements Because many cases of rickets show alternate periods of remission and relapse, secondary to the influence of natural factors or because of variations in treat ment, cases of rickets show various combinations of activity

and healing which at times are difficult to judge from single examinations

The clinical determination of inorganic phosphorus in the serum is a useful test in the diagnosis of rickets and vitamin D deficiency but has certain drawbacks In early or slight deficiencies the values fall in the doubtful range Treatment promptly causes a rise to normal values which do not reflect accurately the progress of the disease, the adequacy of treatment, 'completeness' or speed of recovery, et cetera Furthermore, rickets can and does occur with normal phosphorus values though it is unusual In adults the normal range of phosphate values and the significance of minor variations are not well understood, and mild grades of vitamin-D deficiency and osteomalacia are not reflected by changes in phosphate values Finally, while there is a considerable and constant difference between normal values of adults and infants, in the intermediate period of childhood and adolescence, it is very difficult to interpret the values and tell in a given instance whether they are normal and consistent with age, degree and rate of growth, or abnormal It must be remembered that children at such ages are still growing, and presumably still have a greater tendency to D deficiency than do adults

The generally accepted value for serum inorganic phosphate of infants is 5 0 ± 0 5 mg per 100 cc, with 4 0 mg marking the rachitic zone In rickets values of 2 to 4 mg (sometimes as low as 1 2 mg) are encountered It must be noted that these are in cases of clinical rickets, slight vitamin D deficiency is accompanied by no such variations

In adults the normal level is usually taken to be 2 0 to 5 0 mg, children and adolescents range between the levels of infancy and adulthood It is probable that slight variations occur with variations in rate of growth, and seasonal variations have been observed The latter probably would flatten out if an optimal D was always present In children

and adolescents values for serum phosphate, unless very decidedly low, must be interpreted with caution and in the light of other evidence In them and in adults the determination is of little or no assistance in early or mild deficiencies

Calcium concentration in the blood (serum or plasma) is often determined in cases of suspected vitamin D deficiency and rickets In rickets in infants and children the values are usually normal but may be reduced Normal values in both infants and children are 9 to 11 mg per 100 cc Values as low as 5 mg may be encountered When the serum calcium is low the rickets is apt to be complicated by tetany and in addition to vitamin D there is apt to be an actual deficiency in the intake (dietary) of calcium, or a difficulty in absorption (celiac disease et cetera) In adults the normal values are the same as in children and a decrease in the serum calcium due to vitamin D deficiency is even less common than in children The skeleton of adults provides a store house of calcium and only in severe degrees of deficiency (severe cases of osteomalacia) particularly with an associated calcium deficiency is the serum calcium lowered

Recently the determination of the phosphatase content of the blood has come into favor as a diagnostic test of vitamin D deficiency and rickets The blood phosphatase is elevated in rickets and is thought to be increased before changes in the phosphorus and x ray changes appear Thus it is of value in early diagnosis Furthermore it remains elevated long after treatment is begun, apparently returning to normal only after healing is complete or nearly complete It is thus a more delicate measure of adequate treatment and healing than other procedures Several methods have been devised for determining phosphatase concentration, the results being expressed in various units The method commonly used in this country is that of Bodansky

and the units are called Bodansky units * In infants the normal values are 3 to 12 units In rickets increases are encountered No generally accepted standards for adults are available and the significance of variations has not been well established In a recent survey of a large group of the general population values from 1 5 to 5 0 Bodansky units have been found with a mean of approximately 3 0 units Correlating these values with other studies, including the x ray, serum phosphorus, and calcium, it appears that a value of 3 to 5 Bodansky units is probably normal in adults but the exact significance of variations from this is not clear Furthermore, changes in serum phosphatase values may occur in conditions other than vitamin D deficiency It would appear then that serum phosphatase values are of little help in the diagnosis of vitamin D deficiency in adults at present

In hypervitaminosis D the concentration of both phosphorus and calcium in the serum are increased if the excess is great enough and such an increase may be a forerunner and warning of toxic effect They do not, however, necessarily accompany or parallel toxic symptoms such as nausea, vomiting et cetera

The therapeutic test, as indicated several times before, is of considerable help in the diagnosis of early or mild rickets and vitamin D deficiency, particularly if used with the x ray examination, blood chemistry and in relation to the minor signs and symptoms of the disease In adults this will refer particularly to the pain which seems to accompany the withdrawal of calcium from the skeleton Of course, in some cases (osteomalacia), the effect of treatment on other more gross signs and symptoms is easily demonstrable

In summary then, the diagnosis of vitamin D deficiency will be strongly suspected in infants who present a history of a lack of supplement, will be made in *mild to moderate*

* The method is described in the appendix

rickets and in the osteomalacia of adults by symptoms and physical signs checked by x ray and chemical tests of the blood, may be diagnosed in *early* cases of rickets by the x ray, but the *earliest* cases will be diagnosed by the changes in the blood phosphatase backed by a therapeutic test In adults mild vitamin D deficiency will be difficult to diagnose by any means, but may be suspected by symptoms, x ray changes, and response to treatment Future studies may show that changes revealed by analysis of the blood phosphatase or phosphorus and calcium may be helpful

TREATMENT

The use of vitamin D for the prevention and treatment of rickets is familiar to most physicians but even now not all of the infants who require protection receive it and many cases of rickets are inadequately treated Perhaps this is in part due to the great variety of forms in which vitamin D can be administered, the multiplicity of names, products and units and the confusion which has resulted from rapid advances in our knowledge of this complex substance Much of this confusion will be avoided if vitamin D is prescribed in adequate amounts on the basis of international units

Cod Liver Oil The starting point historically and practically for a discussion of the available forms of vitamin D is cod liver oil Cod liver oil meeting U S P XII requirements contains at least 85 international units* per Gm or approximately 425 per teaspoonful As a matter of fact most cod liver oils contain somewhat more than the U S P XII minimum requirements and several of the better oils contain three or four times as much Most of the vitamin D in

* The international unit is the antirachitic activity of 1 mg of the international standard solution of irradiated ergosterol which is equal to 0 025 microgram of crystalline vitamin D (calciferol) The U S P unit is the same as the international unit except that the U S P reference standard is a cod liver oil which has been assayed against the international standard.

cod liver oil is activated 7 dehydro cholesterol, the type of vitamin D formed in the skin of animals by ultraviolet radiation Cod liver oil, especially the more potent preparations, possesses many advantages among which are the vitamin A it contains, the food value of the oil, its general cheapness, and its availability Its disadvantages are its unpleasant taste, the large bulk if large doses (units) are needed, and the possible danger of so called lipoid pneumonia in infants and feeble patients (by aspiration) Its taste is a minor difficulty because if properly initiated infants will take it readily and will continue to take it well into childhood Its lack of concentration, however, is a serious drawback to its use in treatment when large doses (units) are needed for prompt cure In spite of general opinion to the contrary, cod liver oil rarely causes indigestion Rare cases of hypersensitiveness to it as well as to other fish oils are encountered It may be disguised in various mediums but actually is best given simply from the spoon, perhaps with or followed by the orange juice which is so apt to be a companion part of the infant s dietary protection Finally, the object in giving the oil is to administer the vitamin it contains and if oil cannot be given some other form must be used

Viosterol in oil is the second commonest form of vitamin D Viosterol in oil is ergosterol activated by irradiation with ultraviolet light or bombarded with low velocity electrons and dissolved in some bland oil U S P standards call for at least 10,000 international units per Gm of viosterol in oil and viosterol is so prepared that it is one hundred times stronger than U S P standard cod liver oil The practical measure in usage is the fact that viosterol in oil furnishes *222 units per drop,* with a slight excess to allow for errors in droppers Viosterol in oil offers vitamin D in a concentrated form which allows giving large unit dosage in a form not unpleasant to take and easy to administer Be

cause it is so concentrated it should not be mixed with large amounts of food because of the probability of considerable physical loss on the container Viosterol in oil does *not* contain vitamin A A closely related form is viosterol in propylene glycol, a preparation of pure vitamin D in a solvent which is miscible with water and allows the dose to be mixed with the infant s formula It too lacks vitamin A Both viosterol in oil and viosterol in propylene glycol are plant forms of vitamin D

Fortified Fish Oils A variety of fish liver oils but particu larly halibut liver oil are fortified with viosterol or some concentrated form of D until their potency is equal to that of viosterol in oil Such preparations have the great advan tage of containing vitamin A as well In halibut liver oil the vitamin A content is standardized at about 50 000 I U units per Gm In considering such expressions of potency with respect to vitamin D A or other vitamins it must be remembered that the volume of preparation given at a dose may not be the amount in which unitage is expressed and the unit strength *of the amount given* must be known and kept in mind If fish liver oils are fortified with viosterol the vitamin D is of course activated ergosterol or vegetable vitamin D If reinforced with other potent fish oils it is mainly activated 7 dehydro cholesterol

Concentrated Fish Liver Oils The liver oils of fish of the sub order percomorphi are exceedingly high in vitamin D By combining the oil from these fish of which the blue fin tuna is a good example, in proper proportion a concen trated natural oil is obtained with the same potency as viosterol in oil namely 10 000 units of vitamin D per Gm or 222 units per drop At the same time they contain about 60 000 units of vitamin A *per gram* These provide a highly potent preparation containing both A and D Both fortified and concentrated fish liver oils are available in various forms, in capsules, in bottles with droppers, et cetera Cer

tain concentrates are also provided in tablet form Most of the preparations of concentrated fortified fish oils and viosterol in oil are more expensive than cod liver oil but considered in terms of the amounts actually taken without wastage, spilling et cetera, may sometimes be actually cheaper per unit The form to be used in various circum stances should depend on the physician's judgment

Irradiated cholesterol which, of course, yields activated 7 dehydro cholesterol is available and effective It is the 'animal' form of vitamin D

A variety of combined preparations, vitamin D with carotene, viosterol with dicalcium phosphate, et cetera, are available It should be remembered in using such prepara tions that the important basis for dosage is the number of units required and that at times other added vitamins may increase the cost without adding to the effectiveness of treatment

Vitamin D Foods It is possible by various means to in crease the natural vitamin D content of foods or even to instill vitamin D in foods which do not naturally contain it The chief foods whose content of vitamin D has been sup plied or increased by these means are milk and bread More recently cereals, particularly in the form of breakfast foods, have been similarly treated and eggs are susceptible to an increase of their vitamin D content

Vitamin D milk, so called, is of three kinds, irradiated, metabolized, and fortified Irradiated milk is milk which has been exposed to ultraviolet radiation under certain con ditions which may increase its vitamin D content to as high as 400 international units per quart in some cases The vitamin D is presumably activated 7 dehydro cholesterol Irradiated milk is produced in fresh, dried and evaporated forms

Metabolized vitamin D milk is milk produced by cows who have been fed irradiated yeast, the vitamin D thus being

in the form of activated ergosterol The potency is adjusted to 400 international units per quart The cow herself can be irradiated to increase the vitamin D content of her milk However such treatment can raise the concentration little over that of the natural maximum Actually it only assures this maximum and prevents seasonal decreases

Fortified vitamin D milk is milk to which has been added vitamin D concentrates It too is standardized at 400 international units per quart

Vitamin D bread, made by adding viosterol, is said to contain about 400 units per 24 ounce loaf Obviously the units per slice will depend on the size of the slices Some breakfast foods contain various amounts of vitamin D and their content is usually stated on the container

The difficulty with these vitamin D preparations milk and other foods is twofold they contain so little D that they cannot be relied on to prevent rickets, yet by their use lead to a sense of false security Although capable of preventing rickets it is difficult to adjust the intake of the given article of food to meet the need of both the vitamin and the other factors contained in the food For example milk is a major article of the infant s diet and one which could provide an automatic painless method of providing vitamin D However milk is taken for its calories and other constituents Amounts of milk which are adequate for calories may not provide sufficient vitamin D, while the amounts of milk needed to supply vitamin D may be too great to fit into a well balanced dietary For these reasons the use of such products, while perhaps helpful in the way of general prevention and to assist in providing an added factor of safety, can scarcely be relied upon for the major task of prevention in the most susceptible part of the infant s life Parenteral preparations of vitamin D are not available for clinical use

Note has repeatedly been made of the kind of vitamin D in various preparations, that is, whether the principal part

is activated ergosterol or activated 7 dehydro cholesterol Reference has also been made earlier to the difference in effectiveness of these two forms in animals There is no doubt that such differences in effectiveness exist in respect to certain species and even in man certain differences probably exist Furthermore, it appears that vitamin D is more effec tive in a dispersed form as in milk than in other prepara tions In practice, however, it is safe to consider the two forms of vitamin D practically equal unit per unit if recom mended doses with a margin for safety are followed

Ultraviolet radiations increase the vitamin D available to the body, and can prevent and cure vitamin D deficiency and rickets A variety of sources can be utilized, sunlight itself, carbon arc lights, and mercury vapor quartz lamps All sources are used in prevention and treatment Sunlight in particular is traditionally an important protective source, especially in the case of adults It is a safe and potent agent which should be used liberally Technical difficulties make the use of artificial sources difficult, if not impossible for general use but they may be employed in individual cases and for small groups Many of the lamps emit more ultra violet radiation than sunlight No attempt will be made to give detailed direction for the use of such apparatus Specific technic will vary with the type of apparatus employed In general lamps used by physicians should be sufficiently powerful to produce an erythema in a relatively short ex posure the dosage to be decided upon by the physician in each individual case

Prevention and Protection The greatest need for prophy lactic use of vitamin D is in the protection of special groups General prevention is a relatively simple and unimportant matter Although the exact requirements are unknown, the need of adults and older children appears to be met rather easily by what might be called a normal or usual exposure to sunlight and the amount of pre formed vitamin D con

sumed in the food Lack of such normal exposure and dietary intake automatically puts an individual so affected into a group needing special protection Special protection as in the case of other vitamins ordinarily calls for special supplements of the vitamin

Those requiring special protection are first of all, infants, especially premature and twins, young children, pregnant and nursing women and those who for any reason are confined within doors or do not get a normal exposure to sunlight Lack of exposure is more significant in the latter group if the diet also is faulty and the group includes those individuals who are ill with other diseases invalids, and the aged

By far the largest group requiring special protection are infants and young children In the temperate zone at least this means all infants and young children Theoretically it is possible by strict attention to the feeding of the mother by securing adequate milk at the breast and by making use of all opportunities for exposure to sunlight to rear a child free of rickets without supplements of vitamin D This is especially true in southern latitudes However because of the small margins of safety and the greater need for care and attention which makes success less likely in practice, supplements of vitamin D should be used

With some infants preventive treatment should be begun early in the third or fourth week with 200 international units, increasing in a few days to 400 units and in another week to 800 units where the dose may be held until about the third month when it should be increased to 1200 units for the rest of the first year For the second year it may be reduced to 800 units and it is well to continue this amount for several years All infants should be protected through at least two years and the best practice would probably involve the use of some degree of protection throughout the growing period Premature infants twins and other susceptible

infants, those with gastrointestinal disease or without normal outdoor exposure require greater protection, even to the amount of 5,000 to 10,000 units or more It is better to prevent rickets than cure it and liberal doses in susceptible cases will help insure prevention

Any of the preparations discussed above may be used but irradiated milk and such products as vitamin D bread cannot be relied upon to give adequate protection alone, and for reasons discussed above even metabolized and fortified milk are not entirely suitable Also it would seem best in general to use preparations containing vitamin A which is also apt to be deficient and is a natural companion of vitamin D in fish oils, milk, eggs, et cetera In terms of good cod liver oil, 400 units equals about one teaspoonful and from one half to three teaspoonsful daily will meet the usual requirements from early infancy through childhood For special cases requiring larger unit dosage the more concentrated preparations are advisable In many young infants the certainty of administration and freedom from loss and wastage will justify the use of the more concentrated and expensive preparations The use of such supplements should not exclude exposure to sunlight and vitamin D enriched foods can be employed as an additional supply, or to furnish protection in older children Ultraviolet radiation is very effective and may be used in selected cases, either in the form of solar radiations or from lamps

Pregnant women, particularly during the latter half of pregnancy, and nursing mothers should receive protective supplements, not only for themselves but to protect the infants as far as possible For this purpose moderate doses of 800 to 1200 units per day will be adequate except in special cases This can be supplied by two or three teaspoonsful of a good cod liver oil or equivalent amounts of other preparations Similar protective treatment will suffice in the cases of invalids and others who require protective treatment

Curative For the cure of rickets much more active treatment is required Rickets should be cured rapidly Doses of protective size may cure rickets but the cure may be slow and may permit periods of relapse and irregular progress

Daily doses of 1200 units, or three teaspoonsful of good cod liver oil, will suffice in ordinary cases but larger amounts, such as 5 000 to 10,000 or more units per day, will be required in some cases With the larger doses more concentrated preparations are advisable One should not hesitate to increase the dose drastically if the disease fails to respond Ordinarily about three weeks is required to judge the effectiveness of a given dose or treatment With ordinary dosage the concentration of inorganic phosphorus in the serum begins to rise in about ten days and in three weeks the characteristic signs of healing appear in the roentgenograms Failure of such response is evidence of inadequate dosage and treatment On the other hand mere increase in phosphorus and the appearance of deposits of lime in the x ray film do not mean that treatment is complete Phosphatase concentration is said to remain high until cure is obtained Physical evidence is unreliable because the correction of deformities is very gradual and in mild cases the deformity may be so slight that changes are not detectable The general improvement particularly in muscle function is a helpful indication When the rickets has been controlled the dose can gradually be reduced This will ordinarily be possible in about a month It will be necessary, however, to continue the administration of vitamin D and often in amounts larger than the usual protective doses Some resistant cases require large doses over a long period of time

With very large doses of vitamin D these evidences of improvement described above may appear earlier Ultraviolet radiation is effective and may be used under proper circumstances

Special Cases *Premature* Premature infants often require a larger dose and greater total amounts than full term infants, but usually the larger dosage can be reduced as soon as the period of more rapid development is completed

Refractory Rickets Occasional cases of rickets fail to respond to treatment with even large doses Most of these cases of truly refractory rickets are late rickets and occur in children of three or over In a certain number of them the refractoriness seems to depend on some peculiarity of the individual They are discovered when they fail to respond to the usual treatment Many of these will respond to large doses, the dose being increased until an effect is produced Most cases of refractory rickets will respond when doses in the range of 50 000 to 100,000 units are used but rare cases are encountered in which as high as 1,000,000 to 1,500,000 units daily are required before an effect is obtained Refractory cases usually required larger ' maintenance ' doses

Apparent refractoriness should suggest the presence of another disease and the differential diagnosis between rickets and certain variations of so called endogenous rickets Renal rickets may simulate refractory rickets A syndrome of glycosuria with normal blood sugar level, acidosis due to an unidentified organic acid, dwarfism and an osteoporotic rickets, described by de Toni, Franconi, and others are possibilities as is a rachitic like disturbance associated with a disturbed cystine metabolism and retardation of growth Finally, refractory cases which have responded to the administration of alkali have been reported

Emergency Cases Occasionally in severe rickets the ribs are so weak that the thorax collapses on inspiration respiration is greatly embarrassed, and life is threatened by asphyxia The situation is still more grave if there is an accompanying pneumonia Under such circumstances larger doses, 50 000 units or more daily, should be given and the amount reduced gradually when evidence of healing appears

Rickets and Tetany The treatment of rickets with tetany is the same as the treatment of rickets alone except that calcium should be given with the vitamin D Treatment with vitamin D often causes a temporary drop in serum calcium (which may induce tetany at the beginning of treatment in rickets), and the administration of calcium with vitamin D will prevent an exaggeration of the tetany if it is present Calcium can be given in the form of calcium chloride, calcium lactate, or calcium gluconate but the chloride is somewhat more effective in these circumstances A single dose of three to four grams at first followed by one gram three or four times daily and gradually decreasing after three or four days will usually suffice Ordinarily calcium can be discontinued after a week or two

Vitamin D deficiency in adults is usually mild and responds readily to ordinary doses of the vitamin Even severe cases of osteomalacia if due solely to vitamin D deficiency will respond promptly Doses of 1200 to 1600 units per day or three to four teaspoonsful of good cod liver oil will be adequate in most cases If somewhat more rapid action is desired larger doses may be used but rarely will they prove effective when ordinary doses are ineffective More often failure will be due to an associated but unrecognized deficiency of calcium or to the presence of some disease other than vitamin D deficiency as hyperparathyroidism for example

The treatment of rickets and vitamin D deficiency is specific and ordinarily no treatment is required other than the administration of sufficient vitamin D An exception in the case of rickets is the correction of deformities which may develop in the severe and particularly in the chronic or late cases This is a problem for orthopedic surgery and will not be discussed here It should be emphasized that the proper prevention and treatment of early cases of rickets will obviate the necessity for such corrective surgical procedures

Extensive use of such protection in recent years has greatly lessened the incidence of severe rickets and consequently the number requiring correction of deformities

Toxic Effects of Vitamin D The possibility of toxic effects is almost non existent when the vitamin is used in the treatment and prevention of vitamin D deficiency and rickets Only when the vitamin is given in *massive doses*, as in the treatment of conditions other than rickets or vitamin D deficiency, is the possibility of toxicity a problem Only occasionally in the treatment of refractory rickets or in securing a rapid cure with large doses will the question of toxicity arise in the ordinary treatment with vitamin D When very large doses are given, careful watch should be kept for signs and symptoms of toxicity The symptoms of toxicity appear only some weeks after treatment is started and consist of anorexia, nausea, headache, diarrhea and frequent urination In children pallor and lassitude have been noted With overdosage there may be an increase in calcium and phosphorus levels in the blood, even to pathologically high levels, but it appears that changes in the blood calcium and phosphorus do not necessarily parallel the symptoms The former occur without the latter, and hypercalcemia sometimes develops in the absence of symptoms Nevertheless a hypercalcemia (12 mg per 100 cc or over) should be considered a danger signal With hypercalcemia the urine is apt to be full of calcium crystals and calcium casts may be found If actual severe poisoning with vitamin D occurs and persists *metastatic calcification will undoubtedly occur*

The Use of Vitamin D in the Treatment of Conditions Other than Vitamin D Deficiency or Rickets Vitamin D has been tried and recommended for the treatment of several diseases having, as far as is known, nothing to do with a deficiency of vitamin D These include among others, hay fever, chronic arthritis, and psoriasis In such treatment it is customary to use tremendously large doses, from several

hundred thousand to a million units or more daily It is clear that such treatment has no reference to the use of vitamin D in the relief of a deficiency, but utilizes such pharmacologic properties as vitamin D may possess Such studies have, however, contributed much to our knowledge of the toxicity of the drug and man s tolerance for it, and emphasize the margin of safety between the therapeutic and toxic doses when the vitamin is used for the purpose of relieving a deficiency

In addition to the use of medicinal vitamin D in conditions other than vitamin D deficiency and rickets, ultraviolet irradiations are employed in the treatment of a variety of diseases The situation is even more complex in this case because the ultraviolet radiations exert effects other than increasing the vitamin D content of the body They produce for example erythema and possess bactericidal properties which have no relation to a vitamin D deficiency Nevertheless their use seems to have been based in part on their vitamin D effect as in the treatment of fractures and tuberculosis Discussion of the use of vitamin D in conditions other than those related to a vitamin D deficiency belongs in discussion of the diseases so treated

Calcium Deficiency

(Osteomalacia, Spasmophilia, Infantile Tetany)

HISTORY

A deficiency in the supply of calcium to the body causes very important alterations in the normal anatomy and physiology of the individual Certain of these are quite dramatic as in the case of tetany others more insidious and latent in their clinical expression, are none the less important

Many features of the clinical results of a calcium defi

ciency have been discussed in the previous section on vitamin D deficiency Yet at the expense of some repetition it seems wise to elaborate on the nature of this important mineral and the effect on the body of its inadequacy

In one respect calcium deficiency occupies a unique position among the clinical deficiency diseases now recognized In this condition absolute dietary deficiencies of calcium are less frequent and less important clinically than are the so called conditioned deficiencies In fact it is possible, and frequently proposed, that a dietary deficiency of calcium alone does not occur clinically, and that it appears in adults only when it is conditioned by other nutritional deficiencies or metabolic disturbances

Nutritional calcium deficiencies are recognized clinically as osteomalacia in the adult and spasmophilia in the child (infantile tetany) Infantile tetany has been discussed in the previous chapter There it was pointed out that although calcium deficiency is undoubtedly responsible for several clinical and pathologic features of rickets, tetany is the outstanding expression of a calcium inadequacy in rickets Classical examples of the disease entity osteomalacia are very rare in this country This condition has always aroused considerable interest in medical circles, and, as a result of this interest, numerous studies have been made which clearly indicate the etiological relationship of calcium deficiency to osteomalacia

Celiac disease in children and sprue in adults are the outstanding examples of metabolic disorders which may create secondarily a deficiency of calcium in the body In these two diseases fat is not properly handled by the intestine These fats bind the calcium in the intestine by forming insoluble calcium soaps, and thereby create an inadequacy of the calcium available for absorption and body functions In these disorders of fat metabolism evidences of calcium deficiency are quite common

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NATURE AND FUNCTION

Calcium is one of the mineral elements and is found in the body in much greater absolute amounts than any of the other mineral elements

The great preponderance of the calcium in the body, approximately 97 per cent is found in the bony skeleton The small fraction not deposited in the bones is divided among the teeth blood serum, and the other body fluids such as spinal fluid, joint fluid, and lymph and in comparatively small amounts in the soft tissues of the body In the blood serum (virtually no calcium occurs in the blood cells), calcium may be considered to exist in two states, approximately 45 per cent being in combination with the blood serum proteins (non diffusible) and the other part, approximately 55 per cent, being ionized calcium (diffusible) In other body fluids calcium occurs in the same two forms but in different relative amounts In soft tissues at least a part of the calcium present is combined with tissue colloids

When one considers the nature and function of calcium in the normal individual it is imperative to recognize the mobility of calcium in the body For years it was felt that calcium deposited in the skeleton and teeth was virtually fixed Later work, particularly the recent studies with radioactive calcium, has shown the error of this concept Apparently calcium can be, and constantly is being mobilized from the bones and other tissues into the blood serum Hence, in the event of a chronically deficient calcium intake, the amount of calcium present in the bones and other tissues may be materially and progressively reduced This mobility of body calcium also accounts for the fact that even after long periods of insufficient calcium intake, the serum calcium remains unaltered A lowered serum calcium as is frequently seen in true osteomalacia, occurs only

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when the stores of calcium in the body tissues have been severely depleted

The functions of calcium in metabolic processes are multiple and extremely important ones Since most of the calcium present in the body is incorporated in the skeleton, this function is the most obvious one Calcium constitutes approximately 15 per cent of the weight of fresh bones It occurs in bone principally in two forms, approximately 15 per cent as calcium carbonate and 85 per cent as calcium phosphate The two compounds, together with small amounts of magnesium, potassium, sodium, chloride, fluorine, and iron form a complex compound, and as such give the bone its necessary structural and tensile strength The small fraction of calcium in the body not found in the skeletal system is none the less important functionally This fraction is important in maintaining normal blood coagulability, cardiac rhythmicity, neuromuscular excitability, and membrane permeability These functions are performed principally by the ionized fraction of the serum and tissue calcium When one considers the vital nature of these functions, the necessity of maintaining an adequate blood content of calcium is apparent

Calcium is absorbed from the small intestine, and is made available to body tissue by the blood serum Calcium is constantly being excreted by way of both the urine and feces Normally the urine contains approximately 30 per cent of the calcium excreted and the feces account for 70 per cent The calcium appearing in the feces represents both the unabsorbed intake as well as a portion which was absorbed and then re excreted

Many studies have been made in recent years to evaluate the bodily requirements for calcium The results of various investigators differ slightly, but the following recommended allowances of the Food and Nutrition Board represent good

intakes (allowing for approximately 50 per cent absorption), from 6 months to 12 years, approximately 1 0 Gm, from 12 years to 20 years, 1 0 to 1 4 Gm, adults, approximately 0 8 Gm Pregnancy and lactation cause a considerable increase in the calcium demands and it has been proposed that pregnant women receive 1 5 to 2 0 Gm daily and that lactating women receive 2 0 Gm

PATHOLOGY AND PATHOGENESIS

In considering the pathogenesis of calcium deficiency it is extremely important to remember the interrelation of calcium and vitamin D

It has been definitely established that vitamin D plays a very important role in regulating the absorption of calcium from the intestine An adequate amount of vitamin D will allow a high percentage of the available calcium to be absorbed If the supply of vitamin D is inadequate, only a small fraction of the available calcium will be absorbed Hence it is probable that very small absolute amounts of available calcium may prove adequate if there is a liberal supply of vitamin D And possibly a liberal supply of available calcium may overcome, to a limited degree only, a slight inadequacy of vitamin D When there is an inadequacy of both available calcium and vitamin D, the child will usually develop rickets and the adult will develop osteomalacia Tetany may complicate either condition

Other important secondary factors that influence calcium metabolism have been discussed at length in the previous section These include (1) the absolute amounts and relative proportions of calcium and phosphorus in the diet (2) The availability of the dietary calcium It has been shown that the calcium present in different foods is not equally well absorbed, for example the calcium in milk is well absorbed

whereas that occurring in vegetables is poorly absorbed (3) The reaction of the diet and the presence or absence of hydrochloric acid in the stomach secretions An acid reaction in the intestine favors absorption A diet that increases the alkalinity of the blood when it is burned in the body favors the deposition of calcium (4) Factors other than vitamin D deficiency cause a loss or increased excretion of calcium This is seen in chronic ulcerative colitis, in sprue and in celiac disease Actually the dietary source of vitamin D may also be interfered with in these diseases (5) Finally factors influencing the rate of growth and the general metabolic level are of importance in that they materially affect the body needs for calcium and vitamin D In this respect the importance of pregnancy and lactation in increasing the individual s need for calcium cannot be overemphasized

The pathologic changes resulting from calcium deficiency express themselves principally in the bones, the blood serum, and in the calcium balance of the individual (the balance between the calcium ingested by an individual and that excreted) In calcium deficiency states this balance is always a negative one In other words more calcium is ex creted daily than the subject obtains from dietary sources Obviously this leads to a decrease in the bone content, and if severe enough, to a fall in the blood serum content of calcium Studies on rachitic and osteomalacic bones have shown a definite decrease in the actual bony substance as well as a decrease in the calcium content of this bone Micro scopically one can see an increase in the amount of osteoid tissue present and a decrease in the actual amount of bony substance The result is that there is actually less bone pres ent, and the bone itself is more pliable The pelvis, spine, and extremities are principally affected Obviously such changes lead to bending, and very severe deformities may result When the deficiency reaches the stage of lowered blood calcium, tetany may occur

The pathogenesis of tetany in calcium deficiency states has been shown to be due primarily to the lowering of the calcium content of the blood serum, more specifically to the reduction in the ionized (diffusible) fraction of the serum calcium It has been clearly shown that calcium ions depress neuromuscular excitability Consequently when there is a reduction in the ionized serum calcium, neuromuscular excitability increases and tetanic contractions occur This may become so marked as to cause a generalized convulsion

The relation of calcium deficiency to dental decay has long been a subject of controversy Suffice it to say that it seems that the administration of calcium to pregnant and lactating women is helpful in preventing dental decay Other factors obscure any clear relation of calcium deficiency to dental caries in other individuals

INCIDENCE AND EPIDEMIOLOGY

The frequency and distribution of calcium deficiencies is governed primarily by an inadequate source of vitamin D and by dietary habits supplying insufficient calcium When this combination occurs in children, it results in rickets and is often complicated by tetany Its occurrence in adults is less common due to the fact that most adults obtain adequate supplies of vitamin D and thereby can subsist on minimal amounts of dietary calcium In spite of this occasionally the combination does occur in adults, particularly in elderly people and in those forced to remain indoors constantly for various reasons In such cases mild osteomalacia may result No doubt the general and probably ill advised use of vitamin D therapy for rheumatism was prompted by the occasional beneficial effect when administered to those who do have this mild degree of osteomalacia

Marked osteomalacia with or without tetany, is now a rare condition in Europe and America Endemically it still

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occurs in certain parts of China and the Orient Yet even there it rarely occurs unless the additional demands of pregnancy and lactation are made on the calcium intake Here again we see the extreme importance of conditioning factors in precipitating a deficiency disease In certain religious groups the girls marry at an early age, and thereafter either remain indoors constantly or completely cover their body from light when they do emerge from their homes The additional burden of pregnancy frequently causes osteomalacia in this group

The bony changes and the tetany that occur with sprue and celiac disease have the same incidence and epidemiology as the primary disease This, of course, is rather sporadic in the temperate zones but is much more common in tropical countries

SYMPTOMS AND SIGNS

The symptoms and signs of a mild osteomalacia, such as is occasionally seen in elderly people, are ill defined and not well understood Pain seems to be the principal symptom an aching pain which often is confined to the extremities and is most prominent at night With the pain there may be some general malaise and weakness but the symptoms are too vague to constitute a clearly recognizable picture In the milder forms of this condition, there are no frank helpful signs In the occasional case tenderness may be elicited over the spine

In cases of severe osteomalacia, pain is a very prominent symptom The pain is usually deep seated and aching' in character, is most commonly located in the back and sacroiliac regions, and frequently radiates along the course of spinal nerves The pain is usually unaffected by motion except when pathologic fractures complicate the condition Deformities are the rule in severe osteomalacia They occur

most frequently in the bones of the trunk the spine, pelvis, and thoracic cage The most distressing deformities involve the pelvis, for here they result in a diminution in the size of the bony outlet and constitute a serious complication to pregnancy and labor Muscular weakness, possibly occasioned by the pain, is usually very marked in severe osteomalacia

The symptoms and signs of tetany are well known Latent tetany may be demonstrated frequently by the presence of Chvostek s sign or Trousseau s sign The first consists of striking the facial nerve with a finger or a reflex hammer and obtaining a quick contraction followed by irregular muscular twitches of the facial muscles Trousseau s sign is the obstetric hand contraction often obtained in latent tetany when tourniquet pressure is applied in the region of the elbow An increased sensitivity to the stimulus of galvanic current, Erb s phenomenon also may be elicited in latent tetany Manifest tetany presents the well known picture of carpopedal spasm, tetanic facies and frequently frank convulsions Often laryngospasm and inspiratory apnea occur

DIAGNOSIS

The diagnosis of mild osteomalacia depends upon the history of pain plus a history of an inadequate diet and inadequate exposure to sunlight x ray changes in the bones, and finally the results of a therapeutic trial Obviously the history is only suggestive but even in the milder forms of osteomalacia one can usually demonstrate rarefaction of the bones on x ray examination In such an individual, the administration of vitamin D and calcium causes prompt relief of the symptoms if the diagnosis of osteomalacia is correct

In the more severe cases there is less difficulty in recognizing the condition The pain is usually quite severe and

deformities are often obvious The x ray evidence of rarefaction with bending deformities is striking Examination of the blood serum usually reveals a slightly elevated phosphatase activity and a slightly or markedly lowered calcium content If the serum calcium is sufficiently reduced, tetany will result As has already been stated severe osteomalacia rarely occurs even in those existing on poor diets and inadequate sunshine, unless pregnancy and lactation are added to the burden of a poor nutritional state

Several other causes of osteoporosis must be considered in the differential diagnosis of the milder forms of osteomalacia The severe form with its bending deformities, its lowered serum calcium and its severe pain merely simulates these other conditions Hyperparathyroidism can easily be distinguished by the polycystic bone changes on x ray, by the elevated blood calcium, and by the markedly elevated phosphatase activity Senile osteoporosis does not have such characteristic features but in this condition there is usually no pain encountered unless there are vertebral compression fractures present Actually it is likely that often so called senile osteoporosis is in reality mild osteomalacia The therapeutic trial will readily determine this

Tetany resulting from nutritional inadequacies, may complicate either rickets or osteomalacia as has been pointed out before Its occurrence in these conditions should clearly indicate the proper diagnosis The diagnosis can easily be confirmed by finding a lowered serum calcium in association with a normal or lowered serum phosphorus content The other common types of tetany to be considered in the differential diagnosis are gastric tetany (alkalosis) and hypoparathyroid tetany In the former the tetany is usually the result of prolonged vomiting and a resultant alkalosis Blood examination reveals a normal serum calcium but an increased CO_2 combining power of the blood The tetany

resulting from hypoparathyroidism is rare, usually following operations on the thyroid gland, and is characterized by a lowered serum calcium but a definitely increased serum phosphorus Tetany associated with sprue and celiac disease has the same blood characteristics as rachitic or osteomalacic tetany Again the relation of the tetany to the primary dis ease should indicate the nature of the condition

TREATMENT

The treatment of calcium deficiency states consists merely of the administration of sufficient calcium and vitamin D The *prevention*, *protective*, and *curative* doses of vitamin D necessary for children and adults have been thoroughly dis cussed Calcium may be administered curatively as calcium chloride calcium lactate or calcium gluconate One should remember that the various calcium preparations vary in the content of calcium that is available for absorption Approxi mately 33 per cent of a given amount of calcium chloride is available for absorption, approximately 23 per cent of cal cium lactate and only about 10 per cent of calcium glu conate Therapeutically it is usually desirable to give 1 0 to 2 0 Gm of calcium daily to children and 2 0 to 3 0 Gm daily to adults This will be supplied by 3 to 6 Gm of cal cium chloride or 4 to 8 Gm of calcium lactate in the case of children, and by 6 to 9 Gm of calcium chloride or 8 to 12 Gm daily of calcium lactate in the case of adults Usually the calcium lactate is the therapeutic choice due to the unpleasantness of calcium chloride, and its effect in causing acidosis if administered over a long period of time For preventive and protective purposes the importance of an adequate dietary supply of calcium plus an ample source of vitamin D should be emphasized Ordinarily such a regimen will prove adequate but if for any reason the diet must be

restricted, the calcium preparations mentioned above will be ample provided the vitamin D supply is maintained

It was also pointed out in the previous section that the treatment of tetany requires more energetic measures In this condition large amounts of vitamin D should be ad ministered with both intravenous and oral calcium medica tion Intravenous administration of 1 0 Gm of calcium gluconate should be given two or three times daily, together with calcium by mouth, until the tetany is relieved Fre quently it is necessary to administer acidifying drugs such as ammonium chloride until a restoration of the normal serum calcium content has been attained

In this connection one also might reiterate the danger of precipitating tetany in a case of severe rickets by active vitamin D therapy without supplying an adequate amount of calcium In such instances the rapid removal of the serum calcium to the rachitic bones frequently lowers the serum calcium enough to cause tetany

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8

Vitamin-E (Tocopherol) Deficiency

(Hypovitaminosis E)

HISTORY

EVIDENCE for the existence of vitamin E as an antisterility factor for white female rats was first obtained in 1922 It was later found that the same substance was necessary to prevent testicular degeneration in males No corresponding idiopathic disease had been known in man Sterility has many causes In fact the term antisterility vitamin, used by biologists in referring to vitamin E, is a misnomer in clinical medicine since the term 'sterility is commonly reserved for an inability to conceive The characteristic effect of lack of vitamin E in female rats is to cause premature death and resorption of the fetuses in utero, impregnation and conception occur normally The possible corresponding condition in humans is abortion (habitual) and threatened abortion It is this condition which is thought to be possibly the expression of vitamin E deficiency in man As a matter of fact the evidence that vitamin E is an essential factor for normal parturition in the human is less convincing now than in the past, and the effect of its lack on the male so characteristic and definite in the rat, has not yet been clearly demonstrated in man Recently a relationship to certain neuromuscular disorders has been suggested but this has not been clearly confirmed

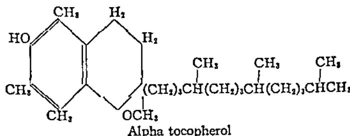
NATURE AND FUNCTION

Vitamin E is a tocopherol, a solid alcohol present in relatively large amounts in wheat germ oil from which it was first isolated It is also present in considerable amounts in vegetable oils, green leaves, and eggs, and in lesser amounts it is very widely distributed in foods It has been isolated in pure form and also has been synthesized There are, however, at least three naturally occurring tocopherals, alpha (α), beta (β), and gamma (γ) tocopherol and several prepared isomers and related compounds which show some vitamin E activity *

Of the naturally occurring forms alpha tocopherol is the most active It is effective in 1 to 3 mg amounts as compared with 5 mg for the beta and gamma forms There is thus a situation analogous to that of certain other vitamins, vitamin D for example, in which several naturally occurring substances ('vitamers) exhibit a similar vitamin activity but with one more active than the others A compound has been prepared artificially which seems to be more active than vitamin E (alpha tocopherol) itself Chemically, vitamin E is related to certain of the sex endocrine hormones

The mechanism by which vitamin E exerts its effects is unknown It is likewise unknown whether it is important in biologic processes in the cells generally or is directly con

* The formula for alpha tocopherol is given in the accompanying figure The structure of beta and gamma tocopherol is the same except that they contain one less methyl group in the aromatic nucleus Chemically vitamin E is related to certain of the endocrine hormones



Alpha tocopherol

cerned only with special organs or tissues Its chemical structure suggests that it may take part in oxidation reduction reactions possibly those concerned with the metabolism of fats Mason s[1] work suggests that it plays an important and essential part in nuclear activities This would explain the particular occurrence of lesions in the developing embryo (and placenta) and in the testes where cellular proliferation and differentiation are proceeding rapidly

It has been suggested by several investigators that deficiency of vitamin E causes a lack of balance between various hormones, especially those of the pituitary and ovary, that influence sex functions Specifically it has been stated that vitamin E is necessary for the normal functioning of the anterior lobe of the pituitary and that a deficiency of the vitamin is in effect a pituitary deficiency Histologic changes in the pituitary gland have been described in E deficient animals Others have reported an excess of estrin like hormones in the blood However no pathologic changes in the pituitary have been reported in humans and the existence of excessive amount of estrin like substances in the blood of women has not been confirmed For the present the nature of such changes in animals, assuming they are due to vitamin E deficiency are obscure and certainly little is known about them in man

Animal studies indicate very little storage of vitamin E in the tissues even after long periods of feeding diets rich in the vitamin Even large doses cannot be accounted for in the urine and following large doses only traces appear Little is found in the tissues except in the body fat Apparently its chemical characteristics are easily changed in the body

PATHOLOGY AND PATHOGENESIS

The pathologic changes of vitamin E deficiency in humans, if any are unknown In animals there is the charac

ter to the last weeks According to some observers there may be an associated toxemia, either early or late Premature and dead or deformed fetuses are not uncommon Reference has been made to a possible relationship between vitamin E and certain neuromuscular disorders, particularly amyotrophic lateral sclerosis and muscular dystrophy Even tabes dorsalis has been mentioned The evidence for such a relationship is the occurrence of paralysis and degenerative changes in the muscles in E deficient animals (rats) and a reported beneficial effect of vitamin E on cases of amyotrophic lateral sclerosis and muscular dystrophy in man So far these beneficial effects have not been generally confirmed

DIAGNOSIS

In the absence of characteristic signs and symptoms or established laboratory tests, the diagnosis of vitamin E deficiency is made by inference and therapeutic trial Shute[2] has developed a test based on a measure of the antiproteolytic *power of the blood This presumably indicates the* balance between vitamin E and an estrogenic substance, but its value has not been confirmed nor has the test been generally accepted As a matter of fact most of the clinical evidence for the theory that vitamin E lack is a cause of habitual abortion in humans is the result of statistical studies on the result of treatment with vitamin E (usually impure in the form of wheat germ oil) In this connection it should be pointed out that the results in some cases are *not definitely* significant, the results are often only slightly better than might be expected with other forms of treatment The diagnosis of vitamin E deficiency in men has not been reported[3] The vitamin is so abundantly supplied in foods *that analysis of the diet is of little or no help* Individual factors which might interfere with the absorption or utilization of the vitamin are unknown

TREATMENT

Vitamin E deficiency, like a deficiency of vitamin K, is a deficiency disease in which there is little need or place for general preventive or prophylactic treatment, the use of the vitamin being confined almost entirely to the relief of specific individual cases under strict medical direction In spite of uncertainty as to its relation to cases of human abortion (sterility) its use seems warranted in selected cases of habitual or threatened abortion when other causes seem to be lacking

Until recently only rather crude preparations have been available and there has been a good deal of variation in the preparations used, their potency their dosage, and even in their exact composition The dosage until the introduction of the pure substance (tocopherol), has been based on the volume of the preparation The preparations most widely used have been wheat germ oil and an extract or concentrate of wheat germ oil The wheat germ oil is given in doses of 4 to 8 cc daily in single or divided doses According to Shute[2] the dosage differs for each patient and tends to increase during pregnancy so that the later treatment is begun the larger the doses needed He also feels that it alters with the season (summer to spring) and with the cyclic phenomena of women The same author feels that an initial saturating dose of as much as 30 to 60 cc may be helpful, and that in occasional cases large doses (90 cc) may be required daily Most other writers have not indicated the need for doses of this size When the plain oil is used care should be taken that it is fresh and potent and that its potency be preserved by keeping it in the cold

Most of the recent treatment has been with concentrates or extracts of the oil using doses of 3 and occasionally 9 minims (0 2 to 0 6 cc) daily in capsules Three minims of

such an extract corresponds to about 5 Gm of the uncon centrated oil and contains about 6 mg of tocopherol Treat ment is begun when the diagnosis is made, or as soon as treatment is decided upon, and is best begun early in preg nancy Treatment should be continued throughout preg nancy Larger doses may be tried in cases of threatened abortion but some consideration must be given to possible untoward effects of large doses (see below) In any case of habitual abortion careful examination should be made for any general or local disease or abnormality which, if found, should be treated or corrected if possible Ordinarily this should be done before one decides to use vitamin E

Certain possible, even though theoretical and unlikely, dangers should be considered in connection with the use of vitamin E It has been thought that spontaneous abortion may be nature s way of getting rid of abnormal products of conception and the wisdom of interfering with it may be questioned The rather significant number of abnormal, de formed, and dead fetuses reported in women delivering after treatment with wheat germ oil (vitamin E) emphasizes this doubt This might be particularly true when treatment is begun late in pregnancy There is also the potential danger when large or massive doses are used, of over inhibiting the protective mechanism of the uterus and leading to excessive chorionic invasion as occurs in the case of hydatidiform mole In this respect it is unwise to administer it in cases of bleeding early in pregnancy without first excluding the possibility of hydatidiform mole Aside from these theo retical but important possibilities no serious untoward effects have been described from the use of vitamin E A few women may show an idiosyncrasy to the oil

Treatment with vitamin E (wheat germ oil) for defective lactation, certain myopathies, tabes dorsalis, carcinoma,

lymphatic leukemia, and for supposed testicular degeneration have been reported but such therapy rests on even less secure evidence than does its use in abortion

REFERENCES

1 Mason E K Differences in Testes Injury and Repair after Vitamin A Deficiency Vitamin E Deficiency and Inanition Amer Jour Anat 52 153 1933
2 Vitamin E A Symposium Held Under the Auspices of the Food Group (Nutrition Panel) of the Society of Chemical Industry April 1939 at the School of Hygiene and Tropical Medicine Keppel Street London W C 1 England
3 *NOTE* Shute[2] has reported finding vitamin E deficiency in 50 to 70 per cent of normal male medical students depending upon the season of the year by his laboratory test
4 The Vitamins A Symposium Arranged Under the Auspices of the Council on Pharmacy and Chemistry and the Council on Foods of the American Medical Association Chicago American Medical Association 1939

9

Vitamin-K Deficiency

(Hemorrhagic Disease of the Newborn, Certain Hemorrhagic States)

HISTORY

One of the vitamins lately shown to play a part in human nutrition and to produce disease when insufficient is vitamin K This vitamin is concerned with the production of prothrombin and a deficiency is followed by a decrease in the prothrombin of the blood If the decrease in prothrombin is great enough various clinical types of hemorrhagic disease appear Because vitamin K affects blood clotting only by its effect on the prothrombin concentration, a deficiency of vitamin K is not a factor in all types of hemorrhagic disease, but only in those in which the bleeding is related to a decreased prothrombin concentration Furthermore, vitamin K is not the only factor concerned in the production of prothrombin severe damage of the liver is one other factor at least which may cause low prothrombin levels and bleeding irrespective of the amount of available vitamin K

Among the hemorrhagic states which have been shown to be related to vitamin K deficiency are the bleeding which may accompany obstructive jaundice, the bleeding associated with diseases and disorders of the gastrointestinal tract such as sprue, celiac disease, ulcerative enterocolitis, et cetera, icterus gravis, and hemorrhagic disease of the

new born Many have been recognized clinically as abnormal conditions of bleeding of obscure etiology, which respond unsatisfactorily to treatment while hemorrhagic disease of the new born is a well known clinical entity Other hemorrhagic states associated with nutritional deficiency may be due at times to a deficiency of vitamin K

The relation of vitamin K to blood clotting and hemorrhage in man was discovered indirectly through research on the metabolism of chicks Dam, investigating the ability of the chick to synthesize cholesterol, found that on a diet which we now know lacked vitamin K the chicks developed hemorrhages Others later made similar observations and the decrease in prothrombin in the blood was found Soon it was discovered that the anti hemorrhagic factor was contained in fish meal, fat of hog s liver alfalfa and other substances Since then progress in the knowledge of this vitamin has been remarkably rapid As late as 1938 its relation to human nutrition and symptomatology was not recognized clearly Since then the vitamin has been isolated in pure form and synthesized, its relation to blood clotting, hemorrhage, and disease in the human has been clearly recognized and described tests for its deficiency have been devised and its use in the prevention and cure of the diseases due to its deficiency have become common place Perhaps nothing else so well illustrates the rapid even breath taking speed of discovery which may occur in the field of nutrition under the drive of modern scientific research

NATURE AND FUNCTION

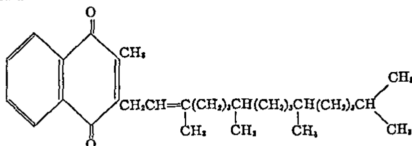
Vitamin K is a substituted derivative of naphthoquinone Like vitamin D it occurs in multiple forms vitamers, at least two natural forms having been isolated one from alfalfa and one from fish meal These are known as K_1 and

K_2 and differ only slightly chemically in the side chain * Like several others of the vitamins, artificial related forms have been synthesized and show the characteristic effect In contrast to most vitamins, however, some of these artificial forms are more potent than the naturally occurring vitamin In addition some of the artificial forms are soluble in water and hence can be more easily employed in parenteral ad ministration

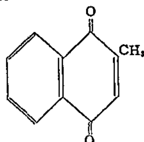
Vitamin K is classed with the fat soluble vitamins Be cause of this characteristic it is to a great degree dependent for adequate absorption on the presence of bile in the in testine This is apparently true to an even greater degree than is the case with the fat soluble vitamin A and carotene

Vitamin K is rather widely distributed in nature in the green leaves of plants such as spinach, cabbage, and cauli flower, in animal fats, and egg yolk It is also present as the result of bacterial action and is probably produced in the

* Vitamin K_1 is 2 methyl 3 phytyl 1, 4 naphthoquinone with the follow ing formula



Vitamin K_2 is very similar A synthetic artificial preparation more powerful than naturally occurring vitamin K recently introduced to clinical use is 2 methyl 1 4 naphthoquinone It has the formula



feces by the bacteria of the intestinal tract This suggests that normally the body is independent of a food supply of the vitamin and that deficiency occurs only when the absorp tion of vitamin produced in the intestinal tract is hindered or prevented This would also explain the drop in pro thrombin in newborn infants before establishment of the bacterial flora of the intestine More recently a few writers have offered evidence indicating that the preformed dietary supply is important and that deficiency may occur from decrease or absence of this portion alone Perhaps both sources are necessary and it may be that some diets fail not only to furnish ready made vitamin K but also fail to pro vide material suitable for synthesis by bacteria in the in testine Further studies will be necessary to determine the relative importance of these sources of supply At present it appears that although the vitamin produced by bacterial action in feces may be an important source of the supply, an exogenous supply from the food may be necessary to a degree that a dietary deficiency may be sufficient to induce symptoms in some cases

Vitamin K is concerned with the production of pro thrombin There is much evidence to indicate that the pro thrombin is formed in the liver but the exact part which vitamin K plays is unknown It is not however, the sole factor and deficiencies of prothrombin occur in spite of an adequate supply and absorption of the vitamin This is seen in cases of severe damage to the liver under which circum stances an excess of vitamin K fails to induce the production of prothrombin With less severe damage large amounts of vitamin K apparently may favor the production of pro thrombin Whether other factors than available vitamin K and a functioning liver are needed is unknown but these seem to be the only two factors of clinical importance recog nized as yet As has been mentioned new born infants seem to be dependent on the supply of prothrombin obtained

from the mother until a supply of vitamin K from bacterial action in their intestine and from food permits the formation of their own supply of prothrombin

Little is known of the general behavior of vitamin K in the body It apparently is stored largely in the liver where it is utilized, but no great storage takes place and deficiency occurs rather quickly if absorption is cut off

In animals an anemia related to vitamin K deficiency has been described but no anemia except that resulting from hemorrhage has been described in man It should be remembered that vitamin K is related to hemorrhage only through its effect on clotting (prothrombin) The hemorrhage itself is due to trauma or other causes (scurvy), the bleeding increasing or persisting to a greater extent than would occur otherwise because of the inadequate clotting

PATHOLOGY AND PATHOGENESIS

The morphologic changes of vitamin K deficiency are those of hemorrhage differing in no particular respect, as far as appearance goes from hemorrhage of other origin The hemorrhage, which is initiated by trauma of greater or less severity and developed by the defective blood clotting presents itself in many forms from petechiae and purpura to persistent bleeding following surgical procedures Many instances of slight, obscure or occult bleeding undoubtedly occur At autopsy hemorrhages in various places are found Blood may be found in the stomach and intestines, areas of petechial and purpuric hemorrhage may be found on any part of the external surface and in the various cavities and viscera A curious finding in a small percentage of patients is ulceration of the stomach or duodenum, sometimes even to the extent of perforation Other pathologic changes are the result of complicating disease

The specific and essential disturbance in the deficiency is functional, a decreased concentration of prothrombin in the blood and hence a prolonged clotting time In the earliest or mildest cases the prothrombin is reduced but not to levels sufficient to interfere with the normal clotting of the blood A decrease in the prothrombin content of the blood can be determined by several methods which constitute an indirect and non specific test for the existence of vitamin K deficiency (See Diagnosis)

Vitamin K is a fat soluble vitamin and dependent for absorption on an adequate supply of bile (bile salts) in the intestine This fact accounts for the mechanism of one of the principal forms of vitamin K hemorrhage the bleeding of jaundiced patients In obstructive jaundice or when there is a failure of the liver to form bile, there is a lack of ab sorption of vitamin K and a deficiency arises in spite of a normal supply in the intestine A similar mechanism is in volved in sprue or celiac disease in which fat absorption may be hindered However other lesions such as intestinal ob struction short circuiting surgical procedures, severe diarrhea et cetera, which lead to deficient absorption gen erally without specifically affecting fat may cause a signifi cant deficiency

In contrast the defective mechanism in hemorrhagic dis ease of the new born appears to be a matter of inadequate supply from the mother The supply is too small to carry the infant over a period of a few days when little is obtained from the bowel because the bacterial flora has not been established and the dietary intake is limited In normal infants there appears to be a natural drop in the prothrom bin level of the blood during this period (2 or 3 days after birth) which does not, however, reach the low level capable of producing hemorrhage In deficient infants this drop is exaggerated to pathologic levels and hemorrhage may occur

INCIDENCE AND EPIDEMIOLOGY

The frequency of vitamin K deficiency is unknown and no attempts to determine its incidence have been made ex cept in a few small selected groups, namely, a limited num ber of new born infants, pregnant women, and small num bers of patients with jaundice and disease of the gall bladder and liver Its occurrence in patients with other diseases likely to interfere wtih intestinal absorption such as sprue, ulcera tive colitis, et cetera, has been reported, also in cases of malnutrition and other deficiency disease No reports of its general incidence have been made but in a recent survey of the nutritional status of several hundred persons I have encountered no cases of these deficiencies

In new born infants a significant number seem to present low prothrombin values, apparently due to vitamin K de ficiency, but there are normal variations in the prothrombin level in different infants and in the same infants at different times Normal standards for these variations have not yet been finally determined, nevertheless, it appears probable that a considerable number of infants have abnormally low prothrombin attributable to a vitamin K deficiency Routine treatment of mother or infant with vitamin K as a prophy lactic measure has been proposed

SYMPTOMS AND SIGNS

The clinical picture of hemorrhage due to deficient pro thrombin has long been familiar to physicians despite ig norance of vitamin K deficiency as a cause Briefly, it can be separated into the three groups previously mentioned, (1) hemorrhage of the new born, (2) hemorrhage associated with disorders of intestinal absorption, most often in jaun

diced states, and (3) hemorrhage associated with malnutrition for example inadequate intake of vitamin K

Hemorrhagic disease of the new born is the most clear cut and distinctive of these various disorders The condition is characterized by multiple hemorrhages occurring in any site (skin mucous membranes of mouth stomach and intestine, brain, lungs or other viscera) varying in extent and location from case to case The bleeding appears to be spontaneous, but in reality is based on trauma though that trauma may be very, very slight and unnoticed Also the bleeding is particularly apt to occur in mucous membranes

The disease is generally considered self limited running a definite course to recovery or death, but in those cases due to vitamin K deficiency which presumably include the great bulk of patients with this syndrome it is apparent that a mild deficiency and tendency to bleeding can persist in a chronic form In most cases, however, the bleeding, which rarely begins before the second or third dav of life appears and is finished by the seventh or eighth day, though occasionally it may be delayed until the tenth or twelfth day Usually the disease lasts but a day or two, death occurring as early as the first day or sooner, only rarely after three days Death results from acute or subacute blood loss or general debility

The symptoms are those of hemorrhage usually visible in the skin and mucous membranes, but many times it first occurs in the stomach or intestines and is noticed when blood is vomited or passed in the stool Blood may be passed in the urine Obviously hemorrhage may occur in concealed areas for example cerebral hemorrhage The rate of blood loss is usually not rapid, but there is a persistent oozing often from surfaces of considerable size Fever may or may not be present, prostration is frequent and diarrhea may develop

Included with these cases of spontaneous hemorrhage

of the new born should be certain cases of bleeding asso ciated with birth trauma While these are it is true, usually associated with injury it is probable that in many cases the bleeding associated with the trauma would have been slight and of little significance had it not been for a diminished prothrombin level in the blood and impaired clotting In fact it is now the belief of some investigators that the ma jority of so called cerebral birth injuries are the result of hemorrhage initiated by trauma but exaggerated and pro longed into extensive bleeding by vitamin K deficiency Other hemorrhages of a similar nature occur elsewhere, hematomas of the scalp, hematomas of the sternocleido mastoid, as well as visceral hemorrhages, but none of these have ordinarily the potential danger of the cerebral (menin geal) hemorrhage No attempt will be made to discuss birth injuries more fully here

Hemorrhage due to disturbances in the absorption of vitamin K is most often seen in association with jaundice but may occur with ulcerative lesions of the gastrointestinal tract, intestinal obstruction, short circuiting surgical pro cedures, sprue or celiac disease, and with severe diarrhea In the absence of jaundice it is familiar as a symptomatic purpura of malnutrition, though extensive bleeding into the skin and hemorrhages from mucous membranes as well as intestinal bleeding can occur In jaundice the disorder is recognized as the tendency to bleed not only in the form of purpura and similar hemorrhages, but also in association with surgical procedures In the latter case the bleeding con stitutes a dangerous and always threatening complication Such a tendency to bleed and actual bleeding is found in patients whose jaundice is due to an interruption in the flow of bile and bile salts into the intestine This occurs in vari ous types of liver failure and in obstruction of the bile ducts from any cause The latter is in most cases, of course, a surgical condition In the past no very satisfactory control

of this tendency to bleeding has been possible, although transfusion which would restore some prothrombin has been partially successful

The signs and symptoms of bleeding due to a vitamin K deficiency arising from an inadequate dietary supply differ in no particular respects from other types of vitamin K hemorrhage except for the associated etiologic factors They are familiar to physicians as the purpura seen with malnutrition and in fact most cases are associated with some other illness which interferes with the intake and absorption of food Thus the factor of absorption is introduced and it becomes doubtful whether they constitute a separate group but rather belong to the group discussed just above Indeed, it may be questioned whether vitamin K deficiency due solely to a diminished dietary intake ever occurs

DIAGNOSIS

The diagnosis of vitamin K deficiency is based on (1) the symptoms and signs of hemorrhage of the types which have been described (2) the presence of disease interfering with the absorption of the vitamin or knowledge of a dietary deficiency (3) a test of the prothrombin content of the blood, and (4) the results of therapeutic trial The dietary history is of little help except in a general way to indicate the possibility of dietary inadequacy The occurrence of bleeding of the types described is highly suggestive and in the case of bleeding of the new born and in jaundiced patients is practically diagnostic This is a late manifestation of the deficiency however and diagnosis if possible, should be made before bleeding develops It must be remembered also that not all cases of bleeding, even under such suspicious circumstances, are due to vitamin K deficiency, and the clinical diagnosis should be checked by other diagnostic tests and a therapeutic trial The test of prothrombin content of the

blood serves to diagnose latent or subclinical instances of the disease and is of particular value in early diagnosis as well as in checking diagnosis made by other means The test is *not specific*, however, because a decrease in prothrombin occurs under other conditions, notably with liver damage, which, if severe enough, leads to a great drop in the prothrombin concentration in spite of adequate or even ex cessive vitamin K intake and absorption The test, therefore, is *presumptive*, but because other causes for hypopro thrombinemia are infrequent and usually easily detected, the test is of great significance Furthermore, it can be, and is, supported by the changes in the test which follow the administration of vitamin K, the therapeutic test Once more it should be emphasized that vitamin K is without effect in hemorrhagic states not associated with reduced prothrombin (hemophilia, thrombocytopenic purpura, scurvy, et cetera)

Several methods for determining the concentration of prothrombin have been devised and used in the experi mental and clinical study of vitamin K deficiency Two of these, the method of Warner, Brinkhous, and Smith, and that of Dam and Glavin are two stage methods of consider able complexity, suited for research purposes but not very practical for ordinary clinical use A third method, that of Quick, is a single stage method which has been widely used both experimentally and clinically By it the amount of *prothrombin is determined from the clotting time of* oxalated plasma after an excess of thromboplastin and a fixed amount of calcium has been added, using a chart pre pared by the author According to this method a normal concentration of prothrombin results in a clotting time of 15 seconds or less A fourth method which is even more simple has been introduced recently by Ziffern, Owen, Hoffman, and Smith By this method the clotting time of a standard amount of whole blood, added to a fixed amount

of thromboplastin is compared with the clotting time of normal blood done simultaneously and the result expressed as a percentage of the normal Thus, if the patient's blood takes twice as long to clot as the normal blood its power to clot is taken to be 50 per cent of normal Values below 70 per cent are considered to indicate a need for vitamin K

All present methods for determining prothrombin content may be criticised because they are indirect Furthermore suitability for clinical use is gained at some loss of specificity and accuracy For example, the test of Ziffern, et al, is simply the comparison of clotting time of whole blood to which has been added some thromboplastin, a process in which many factors are concerned other than the prothrombin level As the authors point out, the end result even as it concerns prothrombin is an expression not only of the prothrombin content but also of the conversion time of prothrombin to thrombin a period which increases as prothrombin supply decreases Nevertheless these tests do measure a disturbance in clotting time the commonest cause of which is decreased prothrombin Other causes for the clotting difficulty can usually be easily determined by other means and the availability for easy frequent, clinical use compensates for some of the defects, provided the tests are made with due understanding and appreciation of their nature the possible errors, and the significance of the results The technic of Quick s test is given in the Appendix

Having established the existence of a lowered prothrombin concentration by these tests the response to the administration of vitamin K as measured by subsequent tests (and clinical signs and symptoms) serves to confirm the diagnosis Except in cases of associated liver damage of severe grade the administration and absorption of vitamin K in adequate dosage will be followed by a rise in prothrombin concentration if the lowered concentration was due to vitamin K

deficiency Even in cases of liver damage, very large doses of vitamin K may enable the liver to raise the prothrombin level to a degree which would be impossible with an ordinary normal supply Such a condition might be termed a rela tive insufficiency though we have not included hypopro thrombinemia due to liver disease in our category of this vitamin deficiency In the absence of hemorrhage, a decrease in prothrombin concentration will indicate a latent or sub clinical deficiency Normally prothrombin is present in such excess that the concentration must fall to 20 per cent of normal before coagulation time is significantly prolonged and below this point before serious hemorrhage occurs This fact has an important bearing on the clinical labora tory tests for the deficiency It is apparent that if a simple clotting time test is done, 80 per cent of the prothrombin must be lacking, and the subject at the bleeding level before the situation is shown by the test On the other hand if one measures the prothrombin content of the blood one can diagnose a latent or preclinical deficiency, and prevent the occurrence of actual hemorrhage These latent de ficiency states are important because they indicate a state of deficiency which may be progressing to a point where bleed ing will occur They are particularly important in the new born and in patients with conditions which might lead to surgery In the former they may reflect a progressive de ficiency which in a few hours might lead to serious or even fatal hemorrhage In the latter the same progressive de ficiency may be present and in addition the loss of blood incident to operation might so further reduce the pro thrombin as to precipitate actual hemorrhage For this rea son the laboratory tests of prothrombin concentration are indicated in those in whom a deficiency might be expected even though no clinical signs of the disease are manifest This is particularly true if such persons are to be exposed to a possible further reduction in prothrombin

TREATMENT

Treatment with vitamin K is highly effective in both pre vention and cure For a time the principal drawback was the cost but this is not very great at present Transfusion, which was the treatment of choice before the discovery of vitamin K, may be used instead of the vitamin or intramuscular injections of whole blood in the prevention of hemorrhagic disease of the new born

In spite of the effectiveness of treatment, there is much confusion in the use of vitamin K because of the rapid progress in the isolation and synthesis of new forms and the rather confusing number and variety of new preparations which have been made available

At present there are available several preparations of vitamin K for clinical use The synthetic preparation 2 methyl 1 4 naphthoquinone has been approved by the Coun cil on Pharmacy and Chemistry of the American Medical Association for inclusion in New and Non Official Reme dies and named menadione Until recently there has not been any practical unit for clinical use The units employed by investigators in this field, of which the Dam unit is a good example, are based on the curative or protective action of a standard substance such as a concentrate of alfalfa against the disease in chicks who have been rendered defi cient artificially With the isolation and synthesis of pure vitamin K and the introduction and use of menadione the units and dosage have been expressed in terms of weight (milligrams) of the vitamin or some closely related com pound At the present time many of the newer synthetic products which are being prepared and offered have their dosage expressed in milligrams

At this time the available preparations are of two general classes, (1) a concentrate of some natural substance (usually

alfalfa) prepared in corn or peanut oil and (2) a pure, syn thetic, artificial preparation also usually put up in oil For the most part the synthetic products are 2 methyl 1, 4 naphthoquinone or derivatives of this substance Most of the preparations are for oral use only but at least one of the artificial products can be given subcutaneously, intramus cularly, or intravenously, and several of the artificial prod ucts in oil have been given intramuscularly with satisfactory results Preparations of vitamin K are available in capsules, tablets, and in bulk solution Those designed for parenteral use are put up in ampules

In ordinary clinical usage, there is no evidence of any toxic effect from the use of this vitamin

All preparations taken by mouth are dependent for ab sorption on the presence of bile salts in the intestine For this reason some form of bile, fresh or desiccated, or bile salts should be given together with vitamin K in all cases in which there is, or is apt to be, a deficiency or absence of bile salts in the intestine Such conditions include obstruc tive jaundice, biliary fistula, and liver damage (conditions which are responsible for a large proportion of the cases of bleeding due to vitamin K deficiency) It is also well, how ever, to include bile salts in cases of intestinal obstruction, faulty digestion of fats, inflammatory disease of the intes tines, et cetera, when poor absorption of K is believed to be *the cause of the deficiency even though there is no jaundice* Theoretically bile alone, by improving absorption, may be adequate to relieve vitamin K deficiency and this has been demonstrated in some cases *However, bile alone* cannot be depended upon to relieve an actual deficit and restore the prothrombin level to normal, at least with the rapidity which is desirable and often very necessary

Preventive Treatment Because of the formation of vita

min K in the intestine by bacterial action and the rather liberal supply contained in an ordinary diet prevention of vitamin K deficiency is not a problem affecting the popula tion generally While the exact daily requirements for man are not known it is probable that they are easily met by the vitamin formed in the intestine and that ingested De ficiency due to lack of natural supply rarely, if ever, occurs and vitamin K deficiency is, in nearly every case, a condi tioned deficiency

Protective Treatment Certain groups of persons clearly need *protective* treatment These are the new born and those with diseases of the digestive system likely to interfere with absorption particularly those with diseases likely to require surgery Because the difficulty is one of absorption rather than supply and requires the administration of large amounts of the vitamin the use of food as a means of pro tection is unsatisfactory Thus in contrast to some of the other vitamins, a concentrated or pure form of the vitamin is needed and protective treatment differs but little from curative treatment

New born infants may be protected by giving vitamin K to the mother just prior to delivery and such treatment has been shown to increase the prothrombin in the infant For this purpose the equivalent of about 1 mg of the commonly employed synthetic vitamin K (2 methyl 1, 4 naphtho quinone) is given daily for three or four days prior to de livery or every other day for the last week If desired a similar amount may be given once weekly for several weeks during the last month of pregnancy as well as at the onset of labor In case the infant is to be protected directly the equivalent of 0 5 mg is given at birth and 1 0 mg daily in divided doses for two or three days These solutions may be placed on the back of the infant s tongue, or later added to the feeding or water In addition to protecting new born

infants against hemorrhagic disease of the new born, infants under one week of age undergoing surgical treatment should be similarly protected

Patients with digestive diseases, whom it is desired to *pro tect* may be given amounts similar to those described for the expectant mother, 1 mg or its equivalent daily However, those cases should also be given bile or bile salts with the vitamin, usually in doses of 0 5 to 1 0 Gm (7 5 to 15 gr) If the patient cannot take these preparations by mouth or cannot retain them, or if the clotting time fails to improve, the preparations should be given by duodenal tube or parenterally For duodenal administration the solution of vitamin K is warmed and instilled through the tube followed by a solution of bile or bile salts in water or physiologic salt solution If preferred, the vitamin preparation and solution of bile salts may be given together but should be kept well mixed

If desired suitable preparations may be given parenterally In the present period of development of these products, it is impossible to give exact doses but in general they are given in the equivalent of one to 2 mg of the pure vitamin to adults and up to 1 mg to children This may be re peated at intervals of six to twelve hours if clotting ac tivity does not improve The effect of such a parenteral in jection usually lasts about a week

If operation is planned such treatment for three or four days preoperatively and for a similar time following opera tion will usually be sufficient as a protective measure If operation is not indicated or is impossible, similar treatment at intervals of every two or three weeks will usually suffice to maintain the prothrombin level

It is assumed however, that the cases under discussion are those in whom no significant drop in prothrombin has oc curred It is further assumed that tests of clotting activity will be made at suitable intervals Should a decrease appear

in spite of this treatment larger doses will be required In these circumstances as much as 5 mg or more may be re quired daily to maintain the prothrombin level Parenteral or duodenal administration and large doses are rarely neces sary for the protection of infants and mothers

At the present time the synthetic vitamin K (2 methyl 1, 4 naphthoquinone) is most commonly used therapeutically The doses in milligrams quoted above represent therapeutic amounts of this drug Usually one capsule or 1 0 cc of the various commercial preparations of 2 methyl 1, 4 naphtho quinone contain 1 0 mg If concentrates of substances con taining naturally occurring vitamin K are used amounts (in capsules or solution form) should be chosen which equal the above mentioned doses in terms of milligrams of the syn thetic vitamin *

Some uncertainty exists as to just how widespread the protective treatment of infants should be Unfortunately, with present methods tests on cord blood are not altogether satisfactory for determining deficiency in the new born Furthermore the infant s prothrombin may be at a border line level at birth and fall to pathologic levels only between the second or third and eighth or ninth days of life, a time which corresponds to the period of bleeding clinically For the majority protection may not be necessary but the possi bility of serious effects particularly if the so called cerebral birth injuries are significantly related to this deficiency justifies widespread treatment for the prevention of even occasional cases For these reasons prevention or protection, particularly that provided through the mother would ap pear to be desirable in the great majority of cases and neces

* The potency of such concentrates is usually expressed in Thayer Doisy Almquist Ansbacher or Dam units For practical purposes of conversion one may consider that 1 0 mg of 2 methyl 1 4 naphthoquinone approxi mately equals 2000 Ansbacher units 1700 Thayer Doisy units 700 Almquist units and 25 000 Dam units

sary in any case which, for any reason, was suspected of being deficient

Curative Treatment When the prothrombin has dropped to a level at which bleeding occurs or when hemorrhage is actually present the deficiency has reached the stage of causing actual disease and treatment is curative rather than preventive. Vitamin K is somewhat unique in possessing a rather sharp quantitative clinical level of deficiency at which actual disease occurs

For infants with actual bleeding or with prothrombin at bleeding levels' prompt treatment is imperative In most cases the same treatment given for protection will be sufficient, namely, 0 5 to 1 0 mg immediately and 1 0 mg daily in divided doses for two or three days

If preferred one of the suitable preparations may be given parenterally in doses of 1 mg of vitamin K (or its equivalent) to be given once or twice daily but the action by the parenteral route is probably somewhat slower It does insure absorption Cases in which the bleeding is not controlled or cases in which the prothrombin level does not rise with these doses should receive larger amounts but most infants respond readily to the amounts indicated

Patients with jaundice and digestive diseases whose prothrombin is reduced to the "bleeding level" or who are already bleeding should be treated promptly with the equivalent of one to three milligrams of vitamin K daily

In the most severe cases the total dose for the first day may be given at once to be followed in twelve hours by the regular usual amounts In all cases it is very important that the prothrombin level be determined at frequent intervals and should the prothrombin concentration fail to rise, larger doses up to four or five milligrams should be given In all cases of jaundice or obstruction of the bile passages, bile or bile salts *must* be given with the vitamin (when given orally) and as previously indicated should probably be given

in all cases in which the deficiency appears to be related to disease of the digestive system The amounts of bile or bile salts range from 0 3 to 2 Gm (5 to 30 gr) according to the amount of vitamin K If difficulty in administration is en countered the vitamin and bile salts may be given in appro priate amounts by duodenal tube as previously described, or by using a suitable preparation it may be given paren terally in doses of one to two milligrams repeated at six to twelve hour intervals according to the response of the pro thrombin Bile salts are not required with parenteral ad ministration

In cases in which operations are performed special care should be taken shortly after operation because even pa tients whose prothrombin has been raised to normal levels may suffer an acute decrease due to dilution of blood and may thereby be reduced to the danger zone In all cases treatment should be continued postoperatively for several days with diminishing doses Patients whose deficit is not relieved by operation or who, without operation, continue to be affected by conditions causing a deficiency must be treated at intervals to maintain the level of prothrombin In a certain number of these however, it might be possible to secure absorption of vitamin K and maintain the pro thrombin level by the administration of bile or bile salts alone If this is attempted, however the prothrombin level should be checked at sufficiently frequent intervals

If the liver is so badly injured that it cannot form pro thrombin vitamin K will be of no help in controlling the hemorrhage associated with the disease However, there is some evidence that a liberal or abundant supply of vitamin K may enable a partially damaged liver to maintain an adequate concentration of vitamin K when otherwise that would not be possible Therefore treatment with vitamin K should be tried even though damage to the liver is present

Furthermore, vitamin K may be given a trial in cases of liver disease not associated with biliary obstruction An alternative treatment of the hemorrhagic states due to vitamin K deficiency is the injection of human blood, either as a transfusion or intramuscularly This treatment has been used with some success in the control of bleeding in new born infants and with patients undergoing surgery for digestive disorders, especially jaundiced patients However, the more powerful and certain effect as well as the ease of administration make the use of vitamin K much superior

Toxic effects from vitamin K used under clinical conditions have not been reported However, manufacturers of synthetic and artificial products warn against the administration of more than two mg daily if given over a period of more than four weeks Also, it is advisable if administration of the drug is continued, to make tests of the red and white cell count and the hemoglobin at frequent intervals

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10

Protein Deficiency

(Nutritional Edema, Hunger Swelling, Alimentary Dropsy et cetera)

HISTORY

NOT ONLY is an adequate amount of protein necessary for health and growth but particular proteins, those containing certain necessary amino acids, are indispensable Not all proteins contain the essential amino acids and all protein is not, therefore equally valuable some merely serves the general need for protein and other forms furnish essential body building stones

In spite of the well known effect of inadequate protein in limiting growth, impairing fertility, diminishing vigor, and similarly affecting the health no clinical syndrome reflecting these abnormalties is recognized These non specific effects rarely occur by themselves Usually they are lost in the gen eral picture of malnutrition or improper growth Clinically protein deficiency is recognized almost solely by a specific effect, a decrease in the proteins of the blood with a resulting loss of osmotic pressure and the development of edema so called nutritional edema

Modern interest in nutritional edema dates from the First World War when the reduction in food supplies in certain countries was accompanied by cases of swelling first among the poor in occupied or desolated areas and in the prison

camps, and later in the general population. However, the disease has been recognized for centuries. Such terms as "famine edema," "hunger swelling," and "prison dropsy," which have been applied to it, indicate the conditions under which it commonly occurred. Although the term "war edema" is relatively new the disease has appeared in epidemic form in many of the recorded wars of history among both the troops and the civil population. As prison dropsy it was endemic in jails and prisons in Europe and America as recently as the last century. Sporadic cases have long been recognized and described under the terms "essential edema," "alimentary dropsy," "anemic dropsy," et cetera. A similar edema occurring in malnourished infants or in those fed principally on a high carbohydrate diet has long been familiar to pediatricians.

It was undoubtedly the astonishing spectacle of a civilized country afflicted in modern times by a famine disease that aroused such great interest in this condition during the First World War. The disease attracted much attention in Germany and Austria when large numbers of cases appeared among the civil population in 1917. Though there was a tendency at first to associate the condition with infections such as relapsing fever, typhus fever, and dysentery, the great interest which was aroused led to numerous studies with the eventual conclusion that the disease was due primarily to a deficiency of food.

These studies also led to the discovery of a hypoproteinemia, the immediate cause of the edema. The full significance of this latter finding was not sufficiently appreciated at first though the importance of a protein deficiency in the diet was early recognized by Rubner, Falta, Schittenhelm, Schlecht and others. Not until after the war was the etiologic significance of the hypoproteinemia clearly understood. This observation, however, contributed to a renewed interest in the whole problem of edema. From it came sup

port for Epstein s ideas of the nature of the edema in nephrosis and such studies as those of Kohman on the rela tion of dietary protein to edema This led to a revival of interest in Starling s hypothesis concerning the exchange of fluid between the blood and the tissues and in turn to other studies which have clarified greatly our understanding of the mechanism classification, and treatment of all forms of edema

NATURE AND FUNCTION

Proteins are very complex organic compounds composed for the most part of hydrogen carbon oxygen and nitrogen and distinguished nutritionally by their content of the latter element Most of the nitrogen of the food and the body is found in the protein In addition to the above elements some proteins contain sulphur, phosphorus and certain other elements in small amounts Although there are numer ous proteins that differ in chemical composition, they all possess the same fundamental characteristics From the view point of nutrition one of the most important of these char acteristics is that they are composed of various amino acids in different combinations

Protein is needed in the diet to build new body protein and replace that lost by wear and tear The body builds its own protein by synthesis from amino acids, combining them in special ways, to form the proteins peculiar to itself It can synthesize some of the amino acids but it obtains most of them from the dietary protein, breaking the latter down during digestion into various amino acids which are ab sorbed and used as building stones to form its body proteins Amino acids which the body cannot synthesize are obviously necessary and indispensable and the proteins which supply them are likewise indispensable Of the 22 amino acids now known 10 appear to be incapable of synthesis by the

body and hence must of necessity be obtained from the proteins of the food Nine of these ten essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophane, valine, and threonine It is probable that cystine, long regarded as an essential amino acid, may be synthesized from an adequate amount of methionine and may finally prove unessential Much is known of some of these amino acids such as tryptophane, cystine, and lysine, the amounts of them in various foods, their biochemical properties, behavior, et cetera About others little is known as yet, and other amino acids may be discovered, some of which may prove to be indispensable

Because some proteins contain certain essential amino acids and others do not, and because the amounts of the amino acids vary in different proteins, proteins differ in their value as food Certain proteins which contain many of the essential amino acids, or large amounts of certain essential amino acids, are said to be of high biologic value, others lacking in these essential amino acids, are said to be of low biologic value In general proteins of animal origin, i e, meat, milk, and eggs are of high biologic value A notable exception is gelatin The proteins of cereals and legume seeds (beans, peas, et cetera) are less complete and in many of them essential amino acids are lacking An example often quoted is zein, the protein of corn, which is lacking in lysine and tryptophane

It should be pointed out, however, that not all the proteins of cereals and legume seeds are thus deficient, some, as the protein of soy beans, may be quite complete In the case of many of the foods containing proteins of poorer value, sufficient complete protein might be obtained provided enough of the food was consumed To accomplish this might, however, necessitate an unduly large intake Also, with vegetable proteins it is usually necessary to secure a wide variety in order to insure a sufficient supply of all the needed amino

acids Proteins of high biologic value, on the other hand, supply large amounts of complete protein in small bulk and taken in any reasonable amount will ensure an adequate intake with respect to the essential amino acids

Proteins are required in the formation of all living cells, in the building of additional cells incident to growth, and in the replacement of cells in the adult Naturally relatively larger amounts of protein are needed for growth This is not the only function of protein, however It is needed also in forming enzymes and hormones, secretions such as milk, and the plasma proteins As previously indicated each tissue each secretion, and each hormone requires a different protein, a particular protein which must have different amino acids in different combinations The immensely wide range of protein necessary to supply the variety of amino acids is, therefore apparent To meet these needs the normal body apparently has, in addition to the daily intake a general protein reserve or depot on which it can draw for proteins for all purposes and which will serve to tide it over periods of deficient intake or abnormal demand Recent studies with isotopes indicate that there is a general pool of nitrogen formed from dietary and reactive body tissue—nitrogen from which protein may be formed for any purpose With more severe deficiency the tissues of the body are drawn on for protein to supply the most necessary purposes, the less important structures being drawn on first In severe deficiency even such important organs as the heart yield some of their protein The protein of the active parenchymal cells of such organs as the liver seem to be affected last Plasma proteins are reduced probably after reserve proteins are exhausted

As described in a previous paragraph protein is not absorbed as such but is broken down into its individual amino acid components then absorbed and transported in the blood to the various tissues for synthesis into body proteins

Digestion takes place mainly in the small intestine under

the influence of a series of proteolytic enzymes Little difficulty is encountered in the digestion and absorption of protein under normal conditions and even in rather severe states of local and general disease Inflammatory and ulcerative disease of the intestine may be severe enough to interfere but often this is as much the result of diarrhea and rapid passage of food as of failure of the digestive and absorptive process Cooking tends to lower the digestibility of some proteins and certain proteins, particularly some from vegetable sources, are digested with difficulty, or not at all These are few, however, and under normal conditions the digestion of protein is easily performed and absorption is quite complete

Presumably the protein used in building the various cells of a tissue is formed at the site of the particular tissue The exact site of deposition of the protein reserve is unknown Protein not required for specific purposes may be burned as fuel or stored as glucose or fat, one third of such an excess being convertible into glucose The rest is broken down and, with the waste protein from degenerated cells and tissues, is excreted in the urine (and stool) as waste nitrogen A large part of the non protein nitrogen of the blood is composed of this waste nitrogen (urea, creatinine, uric acid, et cetera) and as such it appears in the urine Under ordinary conditions in the adult the excretion of nitrogen (waste protein) balances the nitrogen taken in (food protein) and the body is said to be in nitrogen balance Children show a positive nitrogen balance, that is they retain nitrogen (protein) and excrete less than they eat

PATHOLOGY AND PATHOGENESIS

As is the case with the vitamins the protein may be deficient either because the supply in the food is inadequate, because digestion and absorption are deficient or because

of some failure of utilization Deficiency due to failure of utilization is really not a deficiency disease but a metabolic disease, though the ultimate result to the body may be a protein deficiency A deficiency in the supply of protein may be relative or absolute, an abnormally large demand may make a normal supply *relatively* inadequate In com parison with the vitamins the amounts involved are im measurably greater, grams rather than milligrams and *rela tive* deficiencies are more frequent This latter fact is not well appreciated The total caloric intake has an exceedingly important bearing on the adequacy of the protein supply With a high calorie diet the protein intake may be reduced safely to a very low level in the adult (the child requires more but the same principle applies) This is the basis for the satisfactory use of low protein diets by some individuals Under usual conditions a diet of an ordinary caloric value includes a liberal supply of protein, some of which may be burned for fuel When however, the total calories of the diet are reduced more of the protein is burned as fuel and at low calorie levels even normal amounts of protein may be insufficient because so much is diverted in this way from specific use as protein

As already observed most high caloric diets contain lib eral, even large, amounts of protein Adequate low pro tein * diets are ordinarily seen only in dietary experiments or in use by a few food faddists There are some persons whose diets show an absolute shortage of protein, but the protein deficient diet most commonly seen is one low in both calories and protein Practically such a diet results in an *effective* protein shortage even though the amount of protein might be adequate if the calories were sufficiently high Such a deficiency is a *relative* deficiency but it may be just as effective in producing the abnormal changes of pro

* Low protein diets adequate or inadequate may be prescribed by physicians for therapeutic purposes

tein deficiency as an absolute deficiency Clinically most of the protein deficient diets are of this type

Many of the structural and functional changes caused by a deficiency of protein are non specific and are not represented by characteristic changes in either the gross or microscopic picture or in physical signs or symptoms Opportunity for autopsy study of uncomplicated protein deficiency is infrequent, even during epidemics the picture is com plicated by other deficiencies The principal findings which have been reported are a great emaciation with a complete loss of fatty tissue There is atrophy of the heart, liver, and of other tissues but the thyroid gland shows the greatest atrophy of all Effusions into the serous cavities are characteristic Histologically there is a complete absence of fat droplets and a loss of glycogen deposits in the liver These findings suggest that an insufficiency of calories has been present as well as a deficiency of protein I am aware of no autopsy studies on humans in which there was protein deficiency alone

The two most significant findings are the edema and the decrease in the serum proteins There is a decrease in the actual amounts of serum protein in the body as well as a decrease in the concentration The latter may be masked by hemo concentration The immediate cause of the swelling in nutritional edema is the decrease in the serum proteins, a hypoproteinemia This lowers the colloid osmotic pressure of the blood and permits an increased amount of fluid to pass from the blood into the tissues in accord with Starling s hypothesis

The reduction of the serum proteins occurs principally in the albumin fraction, the globulin being normal or slightly elevated in uncomplicated cases and reduced only in the severe cases The colloid osmotic pressure varies with the serum proteins but the concentration of albumin has a much greater effect on the osmotic pressure than the



globulin Not only are changes in the albumin itself of greater importance but there is evidence that the concentration of albumin influences the osmotic pressure effect of the globulin In addition the specific colloid osmotic pressure, i e , pressure per gram of protein, varies with the concentration a gram of protein having a greater osmotic pressure in more concentrated solutions and less in the more dilute

However, in spite of the fact that the relationship between lowered serum proteins and edema is clear in the more severe cases it is less apparent in the milder endemic and sporadic cases, and some uncertainty has existed regarding the significance of the slightly lowered serum proteins in these cases This is due to a failure to appreciate the fact that although a reduction in serum proteins and consequent lowering of osmotic pressure is the primary cause of the edema, there are a variety of secondary factors concerned in its development In the severe cases with a great reduction in serum proteins edema appears irrespective of these secondary factors In the milder cases, with slight or moderate reduction in serum proteins and osmotic pressure, these secondary factors may determine the appearance or non appearance of the edema or, in other words, determine the level of the serum proteins at which edema will occur They also modify the amount of edema when present and influence its disappearance under treatment Among these secondary factors are the intake of salt and water, posture, environmental temperature and tissue tension Salt and water are of course necessary for the formation of edema and within certain limits the giving or withholding of them may cause the edema to appear or disappear or modify the amount of edema The large number of cases of war edema and the frequency of severe cases has been attributed in part to the 'wetness of the diet and the use of large amounts of salt Posture, by affecting capillary pressure through variations in hydrostatic pressure influences the appearance of

edema and its location This factor is responsible for the common observation that the edema usually appears first in the legs in ambulant patients and that the legs are swollen in the evening, the hands and face in the morning It also accounts for the disappearance of edema after a few days' rest in bed in the mild or moderately severe cases and for the failure of edema to develop in bed patients in spite of a reduction in serum proteins to a level sufficient to cause edema in ambulant subjects Variations in environmental temperature by modifying arteriolar and capillary dilatation and hence capillary pressure influence the development and amount of the edema High temperatures are thus responsible for the appearance of 'heat edema' which probably most often has a nutritional background Relaxed tissues permit edema to develop at levels of serum protein which would otherwise not be accompanied by edema, or allow a greater edema to develop Failure to appreciate the importance of these secondary factors is partly due to a false belief in a critical level of serum proteins at which edema occurs Although, under fairly constant conditions, such a critical level may be established statistically in a large series of cases, it cannot be consistently applied to individual cases, because the level of osmotic pressure, though the most important and fundamental factor, is only one of the factors concerned

INCIDENCE AND EPIDEMIOLOGY

Nutritional edema, which may be taken as the clinical expression of protein deficiency, still occurs in epidemics in countries subject to famines such as China and India, and in such countries it exists in endemic form as well It is an ever present threat in any country whose people live near the borderline as far as nutrition is concerned and is to be expected whenever there is a failure of food supplies for

any but a short period Sporadic cases occur in individuals anywhere at any time due to a wide variety of causes Some of these are pure protein deficiencies but many are conditioned, that is, associated with other disease which for one reason or another interferes with the intake or absorption of food Such cases are, of course complicated by the symptoms and signs of the conditioning disease

In this country the deficiency exists in both endemic and sporadic forms Endemic nutritional edema has been reported in certain areas of the South and it is probably present elsewhere among economically handicapped groups Sporadic cases are seen under a wide variety of circumstances from food ' faddism to severe organic disease interfering with nutrition In addition to clinical cases recognizable by the characteristic symptom and sign, edema many milder cases undoubtedly exist many more than are generally realized Many of these perhaps are represented by non specific signs and symptoms, such as subnormal growth and fertility, which cannot be identified in any individual case as due to protein deficiency alone On the other hand the determination of the serum protein concentration, the albumin in particular, may yield evidence of mild protein deficiency before more definite clinical evidence is available Recent studies of this sort suggest that protein deficiency of a mild degree is relatively frequent among certain populations in this country It should be borne in mind however that although the *effective* shortage is in protein, this shortage is often due indirectly to a diet of low caloric value, and hence a protein deficiency (or nutritional edema) of this kind may be an index of general as well as protein nutrition In fact, there is some reason to believe that it is one of the best single measures of general nutrition

All ages are affected and there is no striking difference between the sexes Because of the increased demands of growth, children particularly infants, are apt to suffer more

severely Although in epidemics the incidence is higher in those engaged in hard physical work, the reverse seems to be true in an endemic area, perhaps because the extra food needed is more apt to be provided for the worker Cold and exposure are contributing factors, calling for increased heat production and hence more fuel (calories) which may of necessity be taken from a slender supply of protein Al though most of the endemic cases occur in low income groups poverty is not a necessary factor, and dietary custom and habit may be the determining circumstances Edema, the characteristic expression of protein shortage, is more frequent in warm weather due to the well known effect of environmental temperature on the formation of edema

SYMPTOMS AND SIGNS

None of the signs or symptoms of protein deficiency is in itself specific clinically This is particularly true of such symptoms as fatigability and muscular weakness, or such signs as lessened growth and decreased secretion of milk Even edema, the most characteristic clinical expression of the disease, differs in no particular way clinically from other forms of edema

In practice edema is the essential feature of the disease and in uncomplicated cases is usually the patient s chief complaint Other symptoms occur but are inconsistent and vary with the duration and severity of the disease or depend on the existence of associated disease In sporadic cases asso ciated with other diseases a wide variety of signs and symp toms having nothing to do directly with the nutritional edema may be present

The edema varies from a very slight pitting of the legs to anasarca It is a dependent edema and in the early stages is apt to appear in the legs toward evening, and in the face and hands in the morning when the legs may be free of

swelling Ascites is fairly common in severe cases but hydro thorax and especially hydropericardium are found only occasionally and in the most severe cases The onset is usually gradual but may appear to be abrupt In the latter case a slight edema had probably been present and gradually increasing for some time, a sudden increase being precipitated by an abrupt loss of elasticity of the tissues or a large increase in the edema induced by some unwonted physical exertion or fluid and salt intake I have seen it appear suddenly following unaccustomed walking, and after the drinking of a rather large amount of beer and salt At the onset there is ordinarily only a feeling of slight fulness in the legs or a dull aching Occasionally the pain is more severe and the skin red and hot as though infected Similar symptoms have been observed in patients with 'epidemic dropsy' in India

These are the symptoms and signs in the usual uncomplicated case of nutritional edema, the edema increasing in the more severe cases and such changes as induration, stretching of the tissues pigmentation, et cetera, occurring in the chronic cases Other symptoms and physical signs of less constancy and significance may occur In cases where there has been a reduction in *total* food supply, and especially in epidemics, the first symptom of nutritional edema may be an increase in weight, interrupting a previously steady decline With protein deficiency alone the patient may be well nourished, even obese Weakness and a distaste for exertion are common Work is accomplished with increasing difficulty and there is a loss of efficiency A mild mental depression is frequent An extreme loss of weight may be masked by the edema Except in epidemics the most severe cases are generally sporadic and associated with other disease, gastrointestinal disease particularly

Polyuria and nocturia may be observed if the diet is 'wet but often there is an oliguria with the onset of the edema A slight nocturia, otherwise unexplained is seen in mild

chronic cases The urine and the renal function are normal in uncomplicated cases The same is true of the heart and circulatory system except that the heart may be smaller than normal in severe cases and bradycardia and hypotension are seen with the more severe deficiencies With this there may be an undue tachycardia on exertion and a shortening of breath which seems due to general weakness rather than cardiac failure None of these symptoms or signs are found in mild cases With the circulatory changes in the severe cases there may be a lowered basal metabolic rate and even a hypothermia, but again these are absent from mild cases except that the basal metabolic rate seems to be more often in the lower range of normal

Gastrointestinal symptoms are not ordinarily found in uncomplicated cases and when present are usually the result of the disease which induced the protein deficiency Anorexia and vague indigestion are as much a cause as a result of the deficiency

Neurologic symptoms due to protein deficiency alone are not known and are not found in uncomplicated cases On the other hand mild anemias are commonly observed While many of these are due to other deficiencies, particularly an associated deficiency of iron, the anemia in some cases seems to be due directly to a low protein intake Both microcytic and macrocytic anemias are observed and the relation of the protein deficiency to the anemias is ill defined Occasionally a normal or even high red cell count and hemoglobin content may be due to hemo concentration The values drop to normal or below when normal blood volume is restored Achlorhydria is frequent in patients with nutritional edema but whether it can develop as the result of the deficiency is unknown

In considering the clinical features of protein deficiency, and particularly nutritional edema as a manifestation of that deficiency, it must be remembered that relatively few

uncomplicated cases are encountered In many cases there is a deficiency in calories if not other deficiencies as well, and many cases are induced by other disease and hence many of the signs, symptoms, and other findings may be the result of other causes than a protein deficiency Clinically the edema is the only constant characteristic finding except for the changes in the serum proteins to be discussed below

DIAGNOSIS

Clinically the diagnosis of protein deficiency rests on the presence of an edema associated with a lowered concentration of serum or plasma proteins * or in the earlier cases the finding of a lowered serum protein alone *if the latter is not due to failure to form plasma protein (liver disease) or to loss of protein in the urine or in other body fluids* † As in all deficiency diseases additional evidence may be obtained from a study of the diet and it is easily possible to calculate the intake of protein and determine its adequacy This does not of itself establish actual deficiency however Until body reserves are exhausted an actual clinical deficiency does not exist and in view of the uncertainty in regard to this the diagnosis of a deficiency ordinarily must await some objective evidence such as an otherwise unexplained edema or a lowered concentration of serum proteins A diagnosis of protein deficiency based on disturbances in growth, fertility anemia and the like cannot be made clinically with assurance, nor can diagnoses of deficiencies of specific amino acids Such diagnoses can be suspected strongly if sufficiently accurate knowledge is available as to

* In this book the term serum protein will be used though in practice the protein content of either serum or plasma may be determined There is some difference in the values those for serum being slightly lower

† Nutritional edema with decreased serum proteins may and often does occur with other forms of edema

the diet and particularly if the other evidence of protein deficiency, edema, and low serum proteins, is found

All degrees of reduction in the concentration of serum proteins are found In general the degree of reduction parallels the protein deficiency and the amount of edema Because there is a rather wide range of protein concentra tion in normal subjects the average value has little meaning and the generally accepted minimum level is of greater sig nificance Even these, however, as will be shown below, do not constitute critical levels above and below which edema must be absent and present respectively, nor a conclusive evidence of protein deficiency in itself The matter is fur ther complicated by the fact that the serum proteins are divided rather arbitrarily into two fractions, albumin and globulin, which differ not only with respect to their signifi cance to nutrition but in their effect on osmotic pressure and hence on the formation of edema Finally, there is a difference in the concentration of serum proteins at various ages, and a slight and less significant variation with sex

Many standards have been proposed for serum proteins with certain differences due to methods employed and the number and type of individuals serving as subjects For this country the standards of Peters and Eisenman based on values obtained by the Kjeldahl technic are recom mended [1] According to these standards normal values are as follows Total proteins 6 0 to 8 0 Gm per 100 cc, albumin *4 0 to 5 5 Gm per 100 cc, and globulin 1 4 to 3 0 Gm* per 100 cc

Serum protein values must be considered critically and because of the three parts involved, total, albumin, and globulin, attention must be paid to their relationship For example, because of the normal range of values of each it is possible to have a normal value for albumin and globulin respectively and yet an abnormally low total Thus a low normal globulin of 1 4 and albumin of 4 0 gives a total of

5 4 Gm per cent which is below the standard of normal total serum protein, 6 to 8 Gm per cent In practice such findings are not common but may occur They are not common because when the albumin is at its lower normal levels globulin is usually still well above minimum levels In fact, a total serum protein concentration below normal, with a minimum normal albumin and globulin would probably indicate a true hypoproteinemia in spite of the normal value for the individual fractions

The globulin values are much less significant than those of the albumin, partly because the globulin seems to be less affected by shortage of protein than is the serum albumin and partly because globulin is more often affected, usually *elevated,* by other factors such as infections Sometimes a globulin may be elevated thus to such a degree that an abnormally low albumin is masked in a total protein which is normal This is an example of the need for determining serum protein fractions as well as the total protein

By far the most significant changes in relation to protein deficiency are found in the serum albumin Serum albumin seems less affected by such factors as infection except indirectly through the influence of the latter on nutrition A decrease in serum albumin due to dietary deficiency seems not to occur until body reserves are used up (except in acute hemorrhage) Pathologic increases are rarely found Hyperproteinemia such as is seen in multiple myeloma lymphopathia venereum et cetera, is due to an increase in globulin Low albumin concentrations due to protein deficiency are nearly always associated with a low normal or an abnormally low globulin and hence with a decreased total protein Low albumin with the total protein normal or high due to increased globulin is usually associated wit hother diseases

Considerable importance is sometimes attached to the proportion of albumin to globulin, the so called *albumin*

globulin ratio, and in particular to a *reversal* of this ratio which normally is about 2 5 1 0 Variations in this ratio are

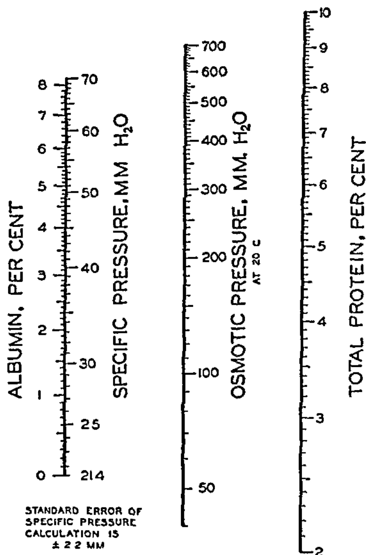


Fig 15 Nomogram A straight line drawn through the proper points on the albumin and total protein scales will intersect the middle scale at the value of the total osmotic pressure calculated from the formula, P = C (21 4 + 5 9 A)

in themselves of little significance unless it is known whether the change in the ratio is due to a decreased or elevated

albumin or a decreased or increased globulin For example a reversal of the ratio due to an abnormally high globulin has a distinctly different meaning than a reversal due to a decrease in the albumin That is, the actual values for albumin and globulin are more significant than the ratio between the two Finally it must be remembered that changes in the serum protein are caused by other things than the dietary supply or absorption of protein

The serum protein concentration is significant, as far as nutritional edema is concerned, only in relation to the colloid osmotic pressure The colloid osmotic pressure may be measured but such measurements are difficult clinically and are rarely made for that purpose Formulae and tables for converting serum protein concentration into colloid osmotic pressure have been devised A formula and nomogram for this purpose are shown in Figure 15 [2]

Numerous standards for the colloid osmotic pressure of the blood plasma or serum have been proposed For practical clinical purposes pressures of 300 to 400 mm of water may be taken as the normal range Edema is often, but not necessarily present, at pressures below 300 mm Because of the difference in the osmotic pressure of albumin and globulin a calculation of the osmotic pressure may be useful in cases in which the albumin alone is decreased and the globulin is sufficiently elevated to give a normal or near normal total serum protein

In summary the diagnosis of protein deficiency while it may be strongly suggested by a knowledge of a dietary deficiency, deficient absorption or the presence of such non specific symptoms as delayed or deficient growth is diagnosed clinically by an otherwise unexplained edema associated with a hypoproteinemia particularly a hypoalbuminemia In early cases it may be disclosed by a hypoproteinemia alone

TREATMENT

Preventive Treatment The prevention of protein deficiency and the resulting nutritional edema is in many cases simply a matter of enough food That is, with adequate calories, sufficient protein is spared so that the amount in the diet becomes sufficient Care must always be taken, however, that a sufficient supply of protein of high biologic value is supplied to afford adequate protection Particular caution should be taken in this respect in certain regions of endemic nutritional edema where custom and habit may dictate diets adequate in total calories but low in protein of good biologic value The same circumstances are encountered among certain food faddists Therapeutic diets are often at fault and should be carefully scrutinized with respect to their content of protein, particularly those which are continued over long periods Very few diseases, however, aside from certain types of nephritis, require any restriction of protein in the diet

The protein requirements may be stated in a general way as amounting to 80 to 100 Gm of total protein per day for the average sized man of average weight engaged in physical activity of average severity Somewhat larger or smaller amounts to suit women and persons of smaller or larger size and weight, more or less active physically will be adjusted automatically by intake if appetite, custom, and availability of food is normal Of the 80 to 100 Gm a certain proportion, perhaps a third or more, in adults, should be first class protein that is, of animal origin (meat, eggs milk, cheese or fish) In the usual American dietary this amount of protein and proportion of animal protein would be furnished by the following Breakfast one large orange, three quarters of a cup of oatmeal with milk and sugar, one boiled egg and three slices of buttered toast Lunch one half cup

Protective Treatment Those requiring special attention or protective care include growing children, pregnant women and nursing mothers, patients with diseases which interfere with the absorption of protein (food), those with chronic infectious diseases, and those in whom demand for protein is increased Protection for the latter group includes those with fever and elevated metabolic rates, not neces sarily because the need for protein is specifically increased but because an increased heat production indirectly raises the requirement for protein Among those with complicat ing disease, particular mention should be made of patients with exudates or transudates that are continually reforming and being removed, and patients with albuminuria The loss of protein in this manner may be very great

The protein requirement for children is distinctly above that for adults, pound for pound, because of the need for growth Desirable daily amounts range from about 4 Gm per Kg (2 2 lbs) of body weight at one year to 2 6 Gm per Kg at six years, after which the amount remains about the same until growth has ceased [3] This is a generally accepted minimum and in no way should be understood to represent the optimum or even desirable amount Of this amount about two thirds should be animal protein

Pregnancy and lactation require a greater supply of pro tein Some animal protein is essential and a good proportion of it should be of this origin Too great a restriction of pro tein has often been made in pregnancy, and sometimes protein has been further restricted because of an edema which originally *was a nutritional edema due to a deficiency of protein* Protein requirements for pregnancy may be placed at the level of non pregnancy during the first trimes ter and increased to a minimum of 1 5 Gm per kilogram of body weight during the remainder of the pregnancy During lactation a minimum of 2 Gm per Kg of body weight is desirable

All surgical patients whose diet has been restricted before operation, particularly those with infectious illnesses, are apt to suffer with protein deficiency and should be properly prepared as far as possible In such patients a nutritional edema, usually appearing postoperatively, precipitated by the salt and water administered parenterally, will often occur unless measures are taken to prevent it Such patients may require even more drastic treatment for prevention namely, transfusion The latter may also be needed in surgical conditions accompanied by a large loss of blood

In concluding the discussion of prevention it should be noted that an actual protein deficiency is rather slow to develop, that the body has a reserve store to tide over periods of reduced intake and sometimes low intakes for considerable periods are *apparently* without effect They do, however, reduce the reserve and once the reserve has been lost the body is particularly sensitive to daily dietary variations in intake Then any deficit is made up by taking protein from more or less vital organs and structures

Curative Treatment The treatment of a protein deficiency which has caused clinical and pathologic changes is also simple and specific and is the same as that required for prevention Certain accessory and symptomatic measures however may be used which will be effective in relieving distress and hastening cure These measures may be of value, when cure by specific measures is delayed or prevented, by restricting or decreasing the edema, preventing stretching of the tissues and the chronic dropsy which may otherwise occur

In uncomplicated cases an adequate diet will suffice to effect a cure Although an adequate caloric intake and a small supplement of protein will be sufficient in many cases it is usually best to give a high protein diet because of its quicker action Animal protein will relieve the edema and restore the serum protein to normal more quickly than

vegetable protein Cases of short duration are apt to be relieved more quickly than chronic cases

Altogether the diet will be *sufficient in uncomplicated* cases Satisfactory treatment may be difficult in cases with gastrointestinal disease which interferes with intake or absorption, in patients subjected to repeated paracentesis, in patients with chronic illnesses, particularly surgical patients, and those with psychotic or psychoneurotic disease

Although the edema itself, if of mild to moderate severity, affects the patient but little, its presence presents a constant threat of the development of such complications as pneumonia, pulmonary edema, infections (erysipelas), and in surgical patients it is probable that the edema is a decided deterrent to wound healing and the recovery of tissues Therefore a prompt relief of the edema and restoration of serum *protein to normal is often desirable In such individuals* the restoration of body proteins and the raising of serum proteins to normal may be hastened by transfusion of either whole blood or plasma Plasma is particularly suited for such a purpose and avoids many of the reactions from transfusions

Another measure for the immediate relief of edema is the use of diuretics The action of these is of course only temporary They are, however, usually effective temporarily and act quickly removing some of the threat of the edema *and preventing continuing stretching of the tissues and the* developing of chronic swelling All of the diuretics are effective but the best are the mercurial diuretics such as salyrgan for immediate effect, and the xanthine diuretics for repeated and continued use When diuretics are used it must be remembered constantly that they are only temporary and palliative, and do not remove the cause of the edema Although some increase in the concentration of serum proteins may be observed following a large diuresis, this is due only to a temporary concentration of the blood,

and the total amount of serum proteins in the body *is not increased* When this temporary concentration is overcome by the intake of water and restoration of blood volume to the previous level the serum proteins will again be diluted, their concentration will fall and fluid will escape from the blood into the tissues, often carrying some of the serum protein with it

In addition to diet, transfusions and diuretics there are a number of simple measures which are very helpful in ridding the patient of edema In mild cases rest in bed for a few days may relieve the patient of edema and rest in bed with elevation of the feet and legs is always helpful Similarly the avoidance of the erect posture, especially quiet standing is of great help

The intake of salt and water has a great influence on the edema and in mild cases a restriction of these alone may cause an edema to disappear or abate This is a particularly important factor in surgical cases Although sodium chloride should not be restricted greatly for long periods its deprivation may be made easier by the substitution of potassium chloride

In those cases in which for various reasons, the edema cannot be relieved and the protein stores restored quickly, elastic bandages and stockings are useful to help prevent the stretching of the tissues and development of a chronic edema which is a possible complication in these cases All of these measures however, other than those designed to restore the intake and body stores of protein to normal are temporary and palliative and should be recognized as such

In a certain number of cases it will be found that though the serum proteins are restored to the normal level the edema persists though usually less in amount This is found most often in chronic cases in which the edema has been present for years Sometimes it is an edema of another type (cardiac usually) but it may be a chronic edema dependent

on a permanent stretching of the tissues In a few cases it may be impossible to restore the serum proteins to normal levels even when there is an adequate intake and no apparent intestinal disease These are usually chronic cases and may be the result of unknown difficulties in absorption or utili zation, the result of a long continued deficiency

Certain of the effects of protein deficiency such as dis turbances in growth appear to respond poorly if at all, once they have occurred, to a relief of the deficiency Neither are they amenable to other forms of treatment

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11

Iron Deficiency

(Iron Deficiency Anemia—Idiopathic Hypochromic Anemia—Iron Deficiency Anemia of Childhood Pregnancy et cetera)

HISTORY

IRON has been recognized as an essential accessory dietary factor ever since its relation to hemoglobin was discovered and long before the rising tide of vitamin research estab lished the modern concept of deficiency disease Following its discovery as a component of hemoglobin the relation of iron to blood formation and anemia was recognized, best perhaps in chlorosis or the green sickness but in other forms of anemia as well Since then the importance of iron as a dietary essential, a factor in the maintenance of normal blood, and in certain cellular oxidation mechanisms, has become increasingly appreciated In consequence, the im portance of iron deficiency anemia as a disease unity as a complication of other diseases (even other anemias) and as the cause of much chronic ill health has been established

Nevertheless despite almost unanimous acceptance of such facts, iron deficiency is curiously neglected in medical practice This neglect seems to be due to the fact that symp toms due to iron deficiency anemia are slight and vague, unless the anemia is great, and physicians fail to realize the frequency of the deficiency as well as the considerable effect even a mild anemia has on the general health There is also a tendency to ascribe anemias to infections or similar

causes, when in fact at least a considerable proportion of such an anemia may be due to an iron deficiency which can be relieved with much benefit to the patient As far as the other functions of iron are concerned there are no known clinical manifestations of a disturbance in them, and so little iron is needed for them that it is doubtful whether a shortage of iron for these functions ever exists

NATURE AND FUNCTION

Iron is one of the elements, a metal, which, since it cannot be synthesized, must be obtained by the body from food and drink or by other means The various combinations in which it is ingested are broken down to release the iron which is then absorbed and recombined in the various forms required for different functions The form in which iron is eaten or administered is of importance only as it affects absorption or in relation to toxic or untoward effects Iron is absolutely essential for the formation of hemoglobin and about one half of the iron in the body (about 6 Gm or 1/5 oz) is normally present in this form However, traces of iron are present in all or nearly all tissues and iron is used in intracellular oxidation as will be described later The remainder is present as the working supply in the process of manufacture or as a reserve store

The combination of iron with protein to form hemoglobin is essential to life Hemoglobin is formed in the blood forming centers where red cells are made It is a very complex compound of high molecular weight consisting of a large molecule, globulin, combined with hematin, an iron containing molecule Although the compound is exceedingly complex all parts of it can apparently be formed or synthesized by the body from simpler substances except iron, which must be supplied directly from the diet or from a reserve previously established Lacking iron from such

sources the amount of hemoglobin which can be formed is found to decrease in proportion to the decrease in the supply of iron

Although the body can synthesize the other constituents of hemoglobin and needs to have only iron supplied as such, the manufacture of hemoglobin is a biologic process affected by other factors such as infections, intoxications, endocrine disturbances, and other dietary factors such as vitamin C deficiency, so that failure to form hemoglobin is not solely the result of iron deficiency Lack of iron is a frequent and important cause, however and often lack of iron complicates other disturbances in hemoglobin formation

Hemoglobin itself is used to carry oxygen to the tissues A small amount of oxygen is carried dissolved in the blood plasma Under certain conditions of oxygen tension in the lungs oxygen and hemoglobin are united in a loose chemical combination oxidized hemoglobin Under conditions of different oxygen tension in the tissues the oxygen is released leaving what is known as reduced hemoglobin Thus hemoglobin without the loosely added oxygen* is reduced hemoglobin (dark) and hemoglobin with the added oxygen is the oxidized form (bright) Because the oxygen carrying capacity of the hemoglobin is almost completely used (85 per cent) under normal conditions increasing the amount of oxygen united to the hemoglobin can only slightly compensate for a loss in hemoglobin Further deficiencies must be met in other ways, such as by faster circulation of blood and more trips per unit of time This induces symptoms of palpitation, tachycardia, et cetera, and may overburden the heart With a severe decrease in the hemoglobin the deficiency is not met and the tissues suffer from oxygen want In addition to this oxygen carrying function hemoglobin also

* Oxygen going to make up the hemoglobin molecule itself is firmly bound and not available for use to the tissues

serves as a "buffer to help maintain the acid base balance of the blood (and indirectly the tissues)

Another function of iron is to assist in the catalytic trans fers of oxygen and hydrogen in the cells These are exceed ingly important, fundamental, and complex biochemical processes but it is very doubtful whether they are ever in terfered with by a lack of iron because the amount of iron needed for this process is very small, 0 3 Gm for the entire body (about 004 mg per Gm of body tissue)

The source of iron is two fold, the mother, from whom the child receives an initial and nonrecurring supply, and the diet The supply from the mother is particularly impor tant because the child depends in a large measure on this supply during a period when it is highly dependent and unable to choose for itself At the same time the demands of growth increase the requirement, while the diet for a considerable period contains naturally a minium of iron Furthermore, as we shall see later, it is difficult to make up deficits of iron from dietary sources alone Therefore the store obtained from the mother is highly important in determining the state of hemoglobin supply during infancy and childhood

The daily requirement for iron varies with age and sex Theoretically, the adult male requires a very small daily intake The average normal adult forms about 25 Gm of hemoglobin daily, requiring about 90 mg of iron This is formed to replace a similar amount destroyed daily How ever, the body is very sparing of its iron and the greater part (at least 85 per cent) of the broken down hemoglobin is recovered and used over again A very small amount of iron is lost in the feces and excretion in the urine is prac tically negligible Therefore, in the adult male, the iron store can be maintained on very small intakes, possibly as little as 1 mg per day under some circumstances (This should not be taken as a recommendation that the intake of

iron be no greater than 1 mg daily) Growth in children and menstruation and pregnancy in women increase the need, yet so carefully does the body conserve its iron that without bleeding or pregnancy, iron deficiency can probably not be induced in an adult if one begins with an adequate reserve Iron deficiency anemia is not produced in animals on diets low in iron unless the animals are also bled or unless one uses young or pregnant animals The clinical significance of this dependency of iron deficiency on bleeding or preg nancy is considerable as will be shown later

The fetus being parasitic, obtains an adequate supply of iron as hemoglobin even at the expense of the mother Unless however, the mother has an adequate intake and store, the iron reserve of the fetus and subsequently of the infant will be deficient Later the increasing demands for growth, occurring at a time of normally poor dietary supply and often improper feeding, will soon exhaust the reserve Anemia then develops This explains the high frequency of iron deficiency anemia in childhood and slight unsuspected anemias of this kind in presumably normal subjects may have influenced the generally accepted normal standards for hemoglobin at these ages As Guest[1] has shown the progres sively diminishing reserve of the mother may be reflected in an increasing anemia of successive children when pregnancies occur at frequent intervals without proper attention to the iron supply and store Such deficiencies unless corrected, handicap the unfortunate child throughout all of its earlier life

The most recent estimates of the daily requirements of the different age and sex groups are based on a rather small number of balance studies in human subjects supported by similar studies in animals In such balance studies the amount of iron lost from the body is balanced against the intake, the amount required to maintain 'balance being taken as the actual requirement In practice 50 per cent is

added to these values as a margin of safety The figures given in Table I represent average safe allowances and suffer somewhat in dependability because of the small number of subjects studied Furthermore, such studies in human subjects are none too reliable because of the variable effect of a reserve store The data are more convincing in animals, in whom the reserve store can be reduced experimentally until the new iron used comes solely from the diet Nevertheless, the figures given may be taken as representing a supply somewhat above that considered to be a safe minimum under normal conditions

The average safe daily allowance for the requirements of a baby up to about one year of age is 0 36 mg per pound (0 9 mg per Kg) In the normal infant this requirement is furnished for the first six months or so, during the time *when the infant s diet (milk) is naturally low in iron,* by reserves provided by the mother Unless the intake is adequate these reserves will be exhausted and anemia will appear at about six months or somewhat later If the reserves are very low it will appear earlier as is frequently the case in twins and premature infants

From about one to three years the safe daily allowance is estimated at 0 27 mg per pound (0 6 mg per kg) For a two year old baby this will amount to 7 or 8 mg For a four year old child 8 mg will serve Increasing demands with increasing weight will normally be cared for by the extra food consumed for that weight provided the child s diet is proper

For boys and girls four to twelve a safe daily allowance is 10 to 12 mg and from 12 to 20 it increases to 15 mg At these amounts the safe allowance for girls may be taken to be about the same as boys though larger amounts have been suggested

For men the daily allowance may be safely placed at 12 mg though some authorities place it at less and it is possible

that less might suffice In view however of the general tend ency to provide liberal allowances for necessary functions and the probable beneficial effect of such allowances, the larger figure may be accepted for general clinical purposes Women, in spite of generally smaller weight should be allowed at least as much as men (15 mg) and it is probably better to set the daily allowance at 20 mg, at least during the child bearing period It must be realized that these allowances do not represent requirements which have been actually and accurately determined on experimental sub jects but are based on such studies and are offered as rea sonable safe working allowances for general clinical use

Safe Allowances for Iron Requirements

Age in years	Sex	Daily allowances in milligram
0– 1	Male and Female	0 36 mg per pound
1– 3	Male and Female	0 27 mg per pound
4–12	Male and Female	8–12 mg (total)
13–20	Male	15 mg (total)
	Female	15 mg (total)
Adult	Male	12 mg (total)
	Female	12–15 mg (total)

Iron, being an ubiquitous element, is found in many foods but like other essential substances it is more abundant in some foods than in others Of equal or perhaps greater im portance than the total amount is the availability of the iron Recent studies have shown that much of the iron in food is unavailable that it is not absorbed or is absorbed very poorly Two factors then influence the dietary supply of iron the amount and the availability Often foods with a smaller total content of iron supply more actual iron to the body because of its relative availability than do foods with a greater total iron content Also, foods which have in the past been accepted as excellent sources of iron have been found to have their iron in an inaccessible form and hence

are comparatively of less value than was formerly thought The content and availability of iron in different foods is discussed under treatment

Absorption and Excretion Iron is absorbed with some difficulty Absorption takes place throughout the gastrointestinal tract but is greatest in the upper small intestine After absorption into the blood, it is carried to, and stored in, the liver and to a less degree in kidney, spleen, and other organs Except for the iron in the hemoglobin of the red cells only small amounts are present in the blood, some 0 1 to 0 3 mg per 100 cc in the plasma Excretion is almost entirely by way of the intestines, but it is unlikely that any but very small amounts are excreted under usual conditions It now seems that absorption is regulated largely by body needs Amounts are absorbed as needed including enough to lay in a rich reserve When this is accomplished absorption lessens and iron passes out unabsorbed Probably little actual excretion occurs

Inorganic iron in all forms is absorbed comparatively well, some better than others True organic iron, not released by peptic or tryptic digestion, is not absorbed and this accounts for the lack of availability of much of the iron in foods Ferric iron before absorption is converted to ferrous iron, a process in which hydrochloric acid assists Normal gastric juice probably favors absorption

PATHOLOGY AND PATHOGENESIS

With this understanding of the requirements for iron, the sources, and the mechanism of absorption, *the pathogenesis of iron deficiency (essentially iron deficiency anemia) is clear* One important fact stands out, namely, that iron deficiency due to dietary lack alone is uncommon and most iron deficiencies are so called conditioned ' deficiencies In the child the deficiency is conditioned by an inadequate

reserve at birth, insufficient to provide the needs of growth at a time when the normal supply of iron in the diet is small The deficiency may be aggravated by a poor diet In the adult the deficiency is conditioned by a loss of iron through hemorrhages, a loss which the usual diet supplies slowly and with difficulty, and for which a diet only slightly deficient in iron may never compensate In women there is the hemorrhage and loss which is physiologic and repeated occasioned by menstruation or pregnancy or both In both men and women a wide variety of conditions may cause hemorrhage and lead to the loss of iron While the loss from a single hemorrhage, even of considerable size may be made up on a good diet, repeated losses of smaller amounts even very small amounts if continued long enough, often may result in a loss of iron which even a good diet cannot supply Finally, various diseases, especially of the gastrointestinal tract, may interfere with absorption and the normal replacement from the diet Many of these diseases are themselves associated with bleeding and iron loss

To summarize then, effective iron deficiency (anemia) rarely occurs in adults without loss of blood* or pregnancy Even children are not apt to develop it if they have an adequate store at birth These conclusions are emphasized by surveys which show a low incidence of such anemias in men but a high incidence in women It is also likely that there is an even greater incidence in women because the standards for women are influenced by the inclusion of mild and unsuspected cases of such an anemia in the supposed normal groups This possibility is supported by the fact that administration of iron to men whose hemoglobin is within the normal range causes little or no increase in hemoglobin while many women show an increase in the concentration

* The author has knowledge of an adult who because of a psychoneurosis subsisted on little but milk for years and developed a severe anemia of the iron-deficiency type without so far as is known the contributing effect of loss of blood

of hemoglobin from minimal "normal' level when given supplements of iron

The gross and microscopic pathology of iron deficiency is that of an anemia The blood and bone marrow show characteristic alterations in uncomplicated cases In a deficiency of sufficient severity the bone marrow shows hyperplasia and there is an increased number of normoblasts The circulating blood shows a hypochromic, microcytic anemia, the characteristics of which are discussed more fully under Diagnosis

INCIDENCE AND EPIDEMIOLOGY

A deficiency of iron in the body, with a resultant anemia, is relatively common As one would expect from the discussion of its pathogenesis, it occurs principally in children and women Iron deficiency anemias in men are decidedly less common, yet they certainly may appear in men, particularly those who are subject to a chronic loss of blood (bleeding hemorrhoids, et cetera) The incidence and distribution of "idiopathic' hypochromic anemia is dependent on an absolute or relative inadequacy of iron in the diet plus the conditioning' factors such as inadequate fetal stores in the child, menstruation and pregnancy in women, and chronic blood loss in any age or sex group Hence it may and does occur in any part of the world Studies on a low economic group in Northeastern Scotland[2] have indicated a 32 per cent incidence of iron deficiency anemia in young children, a 16 per cent incidence in adolescent women, and a 45 per cent incidence in adult women Heath[3] reports that 16 per cent of all the women admitted to the general medical wards of the Boston City Hospital presented evidence of iron deficiency A recent study of an average rural community in middle Tennessee has revealed a significant incidence of iron deficiency anemia in the group

This is particularly important since this study was made on an average community (not a low income group)

SYMPTOMS AND SIGNS

The signs and symptoms of the anemia the clinical manifestation of iron deficiency are the same as those of chronic anemia generally They include pallor, fatigability, weakness, shortness of breath palpitation, tachycardia, vertigo, and syncope Characteristically signs of abnormal blood destruction are lacking (jaundice, enlarged spleen and liver, et cetera) This, however, is the picture of the uncomplicated case Actually iron deficiency is often accompanied by or associated with other diseases Therefore the signs and symptoms of iron deficiency anemia may be overshadowed by those of some disease which itself produces anemia and to which the anemia of iron deficiency is added

In addition to the iron deficiency anemia which occurs as a complication of other disease, a lack of iron produces several more or less distinct anemias clinically These may occur as a complication of other disease They are (1) a hypochromic anemia of infancy and childhood (2) a hypochromic anemia of adolescent girls *chlorosis*, or what corresponds today to chlorosis, (3) a hypochromic anemia of pregnancy, and (4) so called idiopathic hypochromic microcytic anemia To this might be added a hypochromic anemia of chronic blood loss if the term is restricted to those cases of chronic blood loss in which the anemia, rather than the bleeding or cause of bleeding is the prominent clinical feature These conditions differing in exact name and definition according to the classification of anemia used are essentially the same disease Because they are fairly distinct varieties clinically and are usually described as such, the clinical features of each will be discussed separately

Hypochromic microcytic anemia is a common disorder of

infancy and childhood, less common now that supplements of fruit juices, vegetables, fruits, and even meat are added to the infant s milk diet at an earlier age than formerly Factors in the production of the anemia are the large requirement due to the demands of growth, together with either an inadequate endowment from the mother or a diet poor in iron or both Because the maternal supply is a factor, the anemia is more common in premature infants and twins The anemia often appears about the sixth month when the reserves, unsupported by a dietary supply, become exhausted With a poorer endowment of iron from the mother it may appear earlier Or it may not develop until the end of the first year, near the end of the period of rapid growth Other factors such as gastrointestinal disease may aggravate the disorder The child is often plump and appears well nourished but is pale and even bloated in appearance At times such complicating disease as diarrhea may lead to blood concentration and mask the pallor and anemia The blood is characterized by a low hemoglobin content and a normal, high, or low red cell count Usually the red count is reduced and the cells are smaller than normal (microcytosis) and pale Other blood elements are usually unchanged

Iron deficiency anemia does not usually appear after the first year or two of age but anemias developed during infancy are very apt to persist These anemias handicap the child through much of his developmental period This handicap persists because the increasing intake of iron accompanying the greater and more varied dietary, while able to maintain the level of the hemoglobin, cannot make up a deficiency and restore the blood and reserves to normal Treatment with iron is needed to correct this deficiency

The hypochromic anemia of young women corresponds to the chlorosis of former times There is a difference of opinion whether 'true" chlorosis occurs in the present, some maintaining that typical cases are still encountered

(Heath says recently he saw eight in a year) [8] Others maintain that true cases do not occur, though cases much like them as far as the anemia is concerned are not uncommon The crux of the argument seems to be in definition and the difference between present day hypochromic anemia of this type and chlorosis appears to be in such features as the patient s color the bloating of the face, et cetera rather than in the anemia itself It is possible that these latter features were the result of other deficiencies, protein, et cetera which now are not as frequently associated with the anemia At any rate there can be little doubt that the primary feature of chlorosis was an iron deficiency as it is in the hypochromic iron deficiency anemia of girls and young women at the present time

Again the symptoms are those of an anemia, pallor, lassitude, weakness, et cetera, not infrequently accompanied by nervousness and emotional disturbances which may or may not be directly related to the anemia Amenorrhea occurs but is not common in my experience The blood shows a typical hypochromic anemia, similar to that of children, except more often the hemoglobin is *greatly* reduced while the red count remains normal or near normal This leads to an unusually low color index which is considered characteristic

Pregnancy is accompanied by a hypochromic anemia in a large, too large number of women and it is not confined to those in the lower economic levels It is much more common in successive pregnancies Normally in pregnancy there is a drop in the hemoglobin value and number of red cells due to hydremia (increase in blood volume) but this should not blind us to the existence of an iron deficiency anemia It is surprising how often the hemoglobin of pregnant women can be raised by the administration of iron

The clinical picture differs little from that of the other types except that the general feeling of well being so common in pregnancy tends to mask the symptoms of a mild

anemia, while some of the more severe symptoms such as dyspnea, palpitation, et cetera, may be wrongly attributed to pressure from a distended abdomen or even more serious causes The blood itself shows the characteristic changes of iron deficiency, reduced hemoglobin and red cell count, the former more reduced relatively giving a low color index With a good initial store of iron, the hydremia and needs of the fetus can cause only a mild anemia even if the dietary intake is small The severe anemias suggest a prior anemia or some blood loss Poor dietary intake or disturbances in absorption (achlorhydria) have a greater effect in women who are already anemic or whose iron reserves are already low

The so called "idiopathic" hypochromic (microcytic) anemia is an iron deficiency anemia occurring mainly in middle aged women in whom a hereditary or familial constitutional factor has been suspected by some observers This hypothesis has been based largely on the occurrence of achlorhydria in many of these patients and according to some evidence pernicious anemia occurs in these families more often than in the general population Achlorhydria is, however, common in other forms of iron deficiency anemia (perhaps because other deficiencies are commonly associated with iron deficiency) Furthermore, achlorhydria may be a constitutional characteristic of these persons and pernicious anemia may be common in the family, without suggesting that the hypochromic anemia is other than an iron deficiency anemia Achlorhydria hinders the absorption of iron and may contribute to the development of an anemia based *primarily* on a deficiency of iron, while a familial tendency to lack of gastric hydrochloric acid may also indicate a tendency to a lack of the intrinsic anti anemic factor

The important point is that an excessive loss of blood can nearly always be discovered in these women Often it is a chronic menorrhagia or a series of rapidly repeated

pregnancies, and not uncommonly one or more operations, generally for gynecologic disorders Bleeding hemorrhoids may be the source of or contribute to the loss This loss of blood together with a poor dietary intake and impaired absorption (achlorhydria) can account for the anemia in nearly all instances Finally, they respond promptly and completely to the administration of iron Cases are found which resemble these iron deficiency anemias but fail to respond to the administration of an adequate amount of iron Obviously they are not due to iron deficiency However, by far the greater number of cases of anemia similar to those which have been described above are due to iron deficiency

The signs and symptoms are essentially the same as those previously described except for two features One is the occurrence of a change in the nails the so called spoon nails The nails become dry and brittle with longitudinal striations and a turned up free end with undercutting giving a dished or cupped appearance The other is a glossitis and dysphagia which added to the anemia produces the Plummer Vinson syndrome The glossitis resembles that of pernicious anemia with redness and atrophy of the papillae, but the redness is apt to be much greater and of a peculiar scarlet tint which is rather unique Often there is an unexpected lack of pain or soreness The dysphagia is at first unassociated with any objective evidence of obstruction and even in late cases it is doubtful if there are any actual bands or webs of adhesions in the esophagus Characteristically all these signs and symptoms clear up especially on adequate treatment with iron

The hypochromic anemia of blood loss is of course no different essentially from the anemias just described except that in the classifications the blood loss is generally considered to mean blood loss other than that of menstruation and pregnancy, and males are included The bleeding is

usually from some gastrointestinal lesion but may be from other sources The condition may be chronic but such an anemia can follow a single hemorrhage Because it illustrates well the development of an iron deficiency anemia it will be discussed briefly Hemoglobin (iron) lost in a single hemorrhage from a normal person is quickly replaced from the reserve stores and these reserves are replenished gradually from dietary sources If, however, the reserves are too low a hypochromic anemia results This in turn may be relieved by the formation of hemoglobin from iron absorbed from the food How long this takes depends on the adequacy of the diet Even if the diet is good as respects iron, the restoration will be slow Twenty five mg of iron are required for 1 per cent rise of hemoglobin Five mg a day, absorbed and utilized, will raise the level only about 6 per cent in a month The difficulty in raising the level of hemoglobin on poor diets, hampered by interference with absorption and the loss from repeated or continuing bleeding, is readily seen In many cases diet alone, even though high in iron, cannot restore the blood and reserves of iron to normal levels This fact emphasizes the importance of a good reserve store of iron as protection against the vicissitudes of blood loss to which we all are subject

The symptoms of the anemia caused by blood loss of the type just discussed are often overshadowed by the signs and symptoms of the diseases which cause the loss of blood Poor dietary supply and disturbances in absorption are less common than in the other forms The blood itself presents much the same picture as that already described but there seems to be a greater tendency to poikilocytosis However, the clinical signs and symptoms as well as the changes in the blood are so modified by other disease factors that no clear cut picture distinguishes this large group of iron deficiency anemias Even the response to iron is not as helpful in diagnosis because it may be only partial, limited by other factors,

or may occur only when other abnormalities have been corrected or controlled

DIAGNOSIS

The diagnosis of iron deficiency is made by an examination of the blood and the detection of an anemia of the type caused by iron deficiency It may be suggested by the nature of the diet, by the pallor, and by other signs and symptoms of the anemia Confirmation is obtained by the response to adequate treatment with iron Under ordinary circumstances in practice this is manifested by the increase in hemoglobin (and cells), the disappearance of other abnormalities such as microcytosis and if sought for, by the reticulocytosis which follows in response to the iron

Although certain variations occur in the blood picture, mainly quantitatively in the sense of the degree of reduction in cells and hemoglobin on the whole it follows a constant pattern The anemia is above all a hypochromic anemia the primary difficulty is in the building of hemoglobin and this suffers first and most Reduction in the concentration of hemoglobin to 6 or 8 Gm per 100 cc is common and even greater reductions may occur With the loss of hemoglobin there may be a decrease in the number of red cells and in fact, some decrease is nearly always present It is a characteristic of the anemia, however, that the red cells are less reduced in number than is the concentration of hemoglobin and counts of 3 5 to 4 5 million with hemoglobin of 6 to 8 Gm per 100 cc are commonly found In chlorosis essentially normal red counts may be found In cases of greater blood loss however, especially with chronic bleeding the reduction in cells is usually quite severe and may occasionally reach the level seen in such anemias as pernicious anemia In most cases the relation between cells and hemo

globin is such as to cause a low color index, a characteristic feature A color index of 0 6 is frequently encountered

With the decrease in cells and hemoglobin the red cells tend to be smaller than normal (microcytic) The degree of microcytosis varies with the severity of the anemia and while in mild cases there may be little or no decrease in cell size (normocytic anemia) the severer cases show a well marked microcytosis as shown by the hematocrit (mean corpuscular volume) With the decrease in cell size there is not only a decrease in hemoglobin content of the cell (mean corpuscular hemoglobin) but also a decrease in the concentration of hemoglobin per cell, that is the amount of hemoglobin in proportion to the size of the cell (mean corpuscular hemoglobin concentration) Cell size itself may be more useful as an index of the iron shortage and the response to treatment than the cell count and hemoglobin estimation

These changes in the blood may be lacking or obscured in complicated cases In some instances iron deficiency may complicate a macrocytic anemia, even a pernicious anemia In such cases the changes in the hemoglobin content, mean corpuscular hemoglobin and mean corpuscular hemoglobin percentage may not be typical of either type of anemia Other features of the blood picture of iron deficiency anemia also may be altered or lacking under these circumstances

In the blood film the outstanding feature in iron deficiency anemia is the lack of color of the red cells They appear pale and washed out and in some cases almost as empty rings and shadows of normal cells A certain degree of change in shape (poikilocytosis) occurs in the more severe cases and microcytosis can often be detected by simple inspection of the red cells Platelets are normal (or even increased due to bleeding) and, without complication, the white count is normal or slightly reduced (slight leukopenia) The normal proportion of the different varieties of

white cells is maintained unless hemorrhage has induced an increase in granulocytes ('polys) or complications have occurred Reticulocytes are usually normal or low until the increase in response to iron occurs

The differential diagnosis must consider the anemias due to other causes As has already been explained the proper manufacture of hemoglobin in the red cells depends on several factors other than an adequate supply of iron, and failure of other parts of the mechanism may cause an anemia which resembles that of iron deficiency Hypothyroidism of the non myxedematous type and deficient vitamin C may produce such an anemia Lack of protein lessens the formation of hemoglobin even though there is an adequate supply of iron It is doubtful whether deficiencies in the so called trace elements (cobalt, copper, nickel, manganese, et cetera) ever cause an anemia if sufficient iron is present In such anemias the administration of iron will serve to distinguish those which are due to iron deficiency, and the part that is due to iron deficiency when iron deficiency is associated with other forms of anemia Infections, some to a greater extent than others may depress the hemoglobin function as may certain intoxications

TREATMENT

The essence of treatment is adequate dosage Perhaps no other therapeutic pharmacologic substance with the possible exception of digitalis has been the subject of as much constant investigation in the past century as iron Yet the current mistake in the use of iron in insufficient dosage Inorganic iron by mouth is the treatment of choice, organic iron and dietary iron alone are inadequate in the treatment of a real deficiency although adequate iron dietary intake is of prophylactic value Parenteral treatment is rarely indicated and often unsatisfactory Toxic manifes

tations are frequently produced by doses which are large enough to be effective The crux of the question of adequate dosage by mouth is absorption, and optimal absorption demands, among other things, a sufficient concentration Absorption is also favored by the normal secretion of hydrochloric acid in the stomach and is hindered by hypoacidity or anacidity Often a failure of treatment with iron is due to ignorance of, or neglect of, an achlorhydria Lack of acid may to some extent be compensated by larger doses of iron A large number of preparations of iron are available, all of which are effective if given in adequate dosage and anyone of which may, in individual instances, be taken without dis comfort when other forms cause distress The following five official preparations are commonly used and are effective in the daily doses indicated Somewhat larger amounts should be given if no response is obtained Ferrous sulfate, 1 3 Gm (20 gr),* iron and ammonium citrates, 6 Gm (90 gr), pills of ferrous carbonate (pil ferri carbonatis), 4 Gm (60 gr), mass of ferrous carbonate (massa ferri carbonatis), 4 Gm (60 gr), and reduced iron, 3 Gm (45 gr) The first two are freely soluble and may be more effective if there is an achlorhydria The ferrous sulfate has the great advantage of requiring rather small amounts, one 0 3 Gm (5 gr) capsule three times daily for the usual dose The rather low iron content of the iron and ammonium citrate (16 per cent) makes larger amounts necessary It can be given dis solved *in water or other menstruums, or may even* be taken dry followed with water Strong solutions of iron salts should be given by drinking tube to protect the teeth Pills of ferrous carbonate (Blaud s) must be taken in large num bers (7 or 8 a day for an adult) and unless freshly made are

* This dosage is necessary if the ordinary U.S P preparation is used If exsiccated (dried) ferrous sulfate is employed 1 0 Gm daily will be an adequate dose This discrepancy is due to the presence of water in the U S P preparation hence gram for gram it contains about 30 per cent less metallic iron than does the dried ferrous sulfate

apt to be hard and relatively insoluble I have found that reduced iron may be taken without discomfort when other forms of iron cause gastric distress

As indicated iron salts may be taken in solution, in cap sule, or in tablet or pill form Syrups and elixirs are useful vehicles for younger children but care must be taken that *adequate amounts* are prescribed Most official syrups and elixirs of iron contain relatively small amounts These may if necessary be fortified by additional iron In most cases, however, ferrous sulfate will prove as satisfactory in children as in adults All except very young children may be given about the same amount as adults 1 0 Gm (15 gr) daily Very young infants are given 0 6 Gm daily If capsules cannot be swallowed the drug may be added to small amounts of food

The iron should be given in divided doses after meals in order to lessen gastrointestinal disturbance, if nausea, vomit ing or diarrhea occur they may disappear if the drug is stopped for a day and not return when it is started again If iron cannot be taken by mouth 0 1 to 0 2 Gm of green iron and ammonium citrates may be injected intramuscu larly daily but is liable to cause a general as well as a local reaction Iron in organic form is unsuitable because of the large amounts needed As Strauss[5] has pointed out as much as 0 1 Gm of iron ammonium citrates an amount near the toxic level, will provide iron for only a 0 6 per cent rise in the hemoglobin in an adult of average size and 30 such injections would be required to raise the hemoglobin 20 per cent

Diet is effective only in prevention Recent studies have indicated the importance of *availability* of the iron in the diet Many foods, and among them foods reputed to be good sources of iron have the iron in such a form that it is only in small part available to the body In general no more than about 60 per cent of the iron contained in the food

in a general diet can be considered available. The percentage of availability in individual foods ranges from 100 per cent in such foods as apricots, egg yolks, and calf's liver down to 25 per cent or less in beef and lamb meat. Milk is notoriously poor in total iron. Spinach, an often vaunted source, has a high content but much of it is unavailable. Foods relatively rich in iron are: *Liver, kidney,* heart, *oysters,* and *egg yolk,* legumes, leafy vegetables, *nuts,* olives, *whole grain cereals, molasses,* and maple syrup, peaches, *apricots,* prunes, and raisins. Those in italic have a large percentage of their iron in available form.

Prevention. A diet containing liberal amounts of available iron is useful in maintaining a normal store of iron and in replacing it when it has been partially used up. Diet alone, however, cannot replenish a store which has been depleted to the point of producing a significant iron deficiency anemia. For example, a good dietary intake will protect the infant's reserve against the demands of growth, but cannot, ordinarily, supply sufficient iron to cure an anemia resulting from an inadequate endowment reserve from the mother. Similarly, a good dietary supply will restore the reserves after a good sized hemorrhage but the effect of repeated hemorrhages, or one so large as to produce a serious anemia, cannot ordinarily be satisfactorily abolished by dietary iron alone. Therefore, while an adequate dietary intake of iron is necessary to *maintain* our reserves against ordinary depletion, it will not serve for *curative* treatment, nor even for the purpose which we have designated *protection.*

Protection. Protective treatment is indicated for pregnant women, both for themselves and their offsprings. It is also indicated for any person who may be expected to suffer an abnormal loss of blood, particularly if there is reason to expect that the reserves of iron are low. In practice, however, the iron deficiency usually has already reached

the point of an anemia when the patient is seen and treat ment becomes curative rather than protective

Protective treatment differs in no essential detail from the curative treatment described below The principal dif ference is that the smaller doses are sufficient A diet con taining liberal supplies of available iron should be advised Ferrous sulfate in doses of 0 3 Gm (5 grains) four times a day or equivalent amounts of other preparations (see above) should be given For the pregnant woman this may be begun in the latter third of pregnancy and carried out during alternate weeks, alternating perhaps with calcium or iodine or some of the other supplements which are required in pregnancy In other persons similar protective supplements should be given until the danger of a depleted reserve and an anemia has passed

Curative Treatment The cure of an iron deficiency anemia can usually be effected by giving an adequate amount of iron by mouth daily These amounts and the preparations commonly used have already been described Patients should be warned that the medicine may make the stools black (or they may be frightened into omitting the drug) They may also be told that it may cause slight gastric distress but that they should continue with the medicine unless the symptoms are severe

If the patient with what is believed to be iron deficiency fails to respond the following possible explanations and situations should be considered (1) inadequate dosage (2) poor absorption due to achlorhydria (3) disturbance in ab sorption caused by such factors as diarrhea ulcerative dis ease of the intestine, et cetera (4) a loss of blood capable of depleting the hemoglobin (and iron) faster than iron can be absorbed and hemoglobin formed and (5) an incorrect diagnosis

The doses which have been given are on the whole average somewhat above the usual minimal effective dose,

and below the maximum that may be necessary They are, however, considerably greater than those recommended in the pharmacopoeia and, unfortunately, those employed by many physicians Difficulty will be encountered in attempting to increase the doses much above double those given above, though some patients may tolerate larger doses The usual limiting factors are nausea, indigestion, abdominal cramps, et cetera Parenteral administration is not satisfactory as previously pointed out Effective doses (25 mg per 1 per cent rise of hemoglobin) are practically at the level of dosage which produces untoward symptoms

There is considerable doubt whether substances such as cobalt, copper, manganese, etc , which are or may be combined with iron in the formation of hemoglobin, will increase the response to iron if the latter is given in adequate amounts (This does not refer to action of such substances as thyroid extract and vitamin C) There is some evidence in animals of a beneficial effect but clinically there is little evidence that they will add to the effectiveness of iron in adequate dosages Copper may occasionally aid, especially in infants

Achlorhydria may affect absorption unfavorably and prevent a proper response to iron The administration of hydrochloric acid in adequate doses may improve absorption to the point where the dose of iron is effective (Some authors do not believe that hydrochloric acid or the lack of it significantly affects the absorption of iron) Gastric analysis is recommended prior to treatment with iron

When the difficulty of absorption is due to other gastrointestinal disease the latter is usually apparent, though occasionally failure to respond to iron may suggest some lesion and lead to its diagnosis *Difficulties in absorption of this* kind can usually be overcome with adequate dosage and with proper treatment of the underlying disease

In the case of bleeding where hemoglobin is lost faster than it can be restored, no treatment with iron will be effec

tive though it should be given in large doses to maintain the blood at as high a level as possible Treatment consists in the control of the bleeding followed by adequate amounts of iron and such other treatment (transfusion) as is indicated

There remains the question of a mistaken diagnosis The response to iron may often be taken as a test of the diagnosis This is best done by examining the blood for the reticulocytosis which accompanies a rise in hemoglobin due to the administration of iron Failure to observe such a reticulocytosis, which is as specific but not usually as great as in pernicious anemia is strong evidence against an iron deficiency anemia and a prophecy of failure in increasing the hemoglobin and red cells Increase of hemoglobin and red cells means that the reticulosis was overlooked or that some other, unrecognized factor has acted In addition, study of the reticulocytes is a good check on dosage often a reticulocyte response will not appear until the dose has been increased to an adequate amount In considering diagnosis however and the reticulocytosis and increase in hemoglobin and cells it must be remembered that iron deficiency may exist with other kinds of anemia and that the response to iron may be limited by other factors than the supply of iron itself

A method of treatment which may succeed when the iron alone fails is transfusion This is a common method of treatment of anemia due to loss of blood surgically but is equally good, though neglected, in other forms of severe or stubborn iron deficiency anemia A common mistake, however, is to neglect to give iron with and following the transfusion The latter helps greatly but often does not cause complete restoration of hemoglobin and cells

Finally, it is necessary to emphasize the necessity and importance of complete cure of treatment which will raise the blood to completely normal, even high normal levels It is not sufficiently appreciated how much even a *slight* anemia

affects the well being and good health, or how common such a slight deficiency is and how much a part it plays in the lives of countless persons, especially women All too often treatment is stopped before cure is complete This is particularly true of surgical and gynecologic patients The patient is admitted for an operative procedure, and is often already anemic She may be given a transfusion before operation and often a transfusion after the operation which itself has entailed further blood loss With this iron may or may not be given Such treatment *improves* her anemia but there, too often, treatment is stopped The blood is raised toward normal, or perhaps even to low normal levels but treatment is not maintained until *full recovery* is secured The reserves are not built up and the patient is apt to "stick" at a point of mild iron deficiency anemia, handicapped by failure to secure a complete cure of the anemia Only careful conscientious treatment will prevent this unnecessary and unfortunately too common failure to relieve completely a deficiency disease

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12

Iodine Deficiency

(Simple goiter—Cretinism)

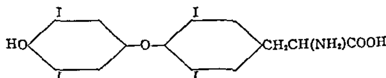
HISTORY

Goiter and cretinism due to a lack or shortage of iodine in the food or water is as truly a deficiency disease as that caused by the lack of a vitamin Iodine is a substance essential to normal growth, development, and health which cannot be synthesized by the body and must be obtained from without Goiter, being a physical abnormality and easily visible except in its mildest forms, has been known and recognized from time immemorial by laymen as well as physicians So too has its relation to iodine been recognized more or less clearly for centuries, though at times this relationship has been lost from sight The occurrence of goiter and cretinism in endemics and epidemics, the recognition of endemic goiter regions and the variations in the incidence of goiter and goitrous disease in different parts of the world are all long standing matters of common knowledge In this country iodine deficiency, and consequently endemic goiter, is found in several definite regions whereas sporadic deficiency, relative or absolute, depending on individual circumstances may be encountered anywhere Nowhere in this country, however, do areas of such severe iodine deficiency, or intense goitrousness,' exist as in some parts of Europe and Asia such as Switzerland or the Himalayas

NATURE AND FUNCTION

Iodine is a chemical element widely distributed in nature but for the most part, especially in relation to food and drink, occurring in such small amounts as to fall into the general classification of "trace" substances In the body, iodine s primary, if not sole, function is to combine with the amino acid, tyrosine, to form thyroxine, the active principal of the thyroid gland * This in turn is united with a globulin to form thyroglobulin which is the form in which the hor mone is stored in the thyroid gland and presumably secreted for use by the tissues In addition to that found in thyroxine, part of the iodine is found in diiodotyrosine, apparently an intermediate substance occurring in the formation of thy roxine Also, some *free* iodine is found in the thyroid gland apparently the raw material from which thyroxine is formed The principal body store of iodine is in the thyroid, the gland absorbing the iodine from the blood and storing it as raw material or as the partial or completed hormone in the colloid The remaining portion of the iodine in the body occurs either as thyroxine performing its function in the tissue cells, or in the process of transport in the blood, or is present as free iodine Once the store house in the thyroid gland is full, any excess iodine is excreted in the urine, feces, tears, milk, et cetera and has significance only as a chemical with such properties as it is known to have pharma cologically Even when large amounts are administered as a drug, any excess beyond the exceedingly small amounts

* Thyroxine has the formula



needed by the thyroid is inactive in the normal metabolic processes of the body

The amount of iodine needed for the formation of thyroxine and the normal metabolic purposes is exceedingly small The maximum storage in the thyroid gland is about 25 mg and the normal content averages around 9 to 10 mg Rather wide variations are encountered (as great as from 0 05 to 0 45 per cent (dry weight) but the range in disease is still larger Iodine in active use in the body in the form of thyroglobulin probably totals about 20 mg with some 0 6 mg in the blood Maximum daily amounts needed to maintain the circulating thyroxine and stores in the thyroid are estimated at about 0 2 mg The daily requirement under ordinary conditions is much less than that, indicating that as thyroxine is broken down the iodine freed is carefully and economically salvaged by the thyroid gland to be used over again in the formation of new thyroxine The daily requirement has been estimated at 0 02 to 0 07 mg (20 to 70 gamma) being relatively greater in children

Thyroxine is a catalyst affecting the metabolism of the tissue cells presumably all the cells Its effect is to increase the metabolism and to maintain it at a higher level than would be maintained if thyroxine was not present

The usual source of iodine is the water and food ingested The iodine content of those depends on the iodine content of the soil on which the food is produced and through which the water passes Soils show wide variations in their iodine content on the basis of geologic and geographical characteristics Certain areas are extremely deficient in iodine and in them a deficiency of iodine in the food and water will exist In other regions, sea coasts for example the soil and water may be rich in iodine These variations in the iodine of the soil however, cannot always be directly correlated with the iodine intake of inhabitants of given regions A central water supply may be drawn from a region where the

iodine in the soil is much less than it is in the area where the water is consumed On the other hand, in an area of soils with little iodine most of the food actually eaten may be imported from regions where the iodine supply is ade quate This modern diversity of food sources is probably a factor in the decreasing incidence of endemic goiter An older but even more striking instance of the effect of 'for eign food is the consumption by inlanders of marine prod ucts rich in iodine such as fish, shell fish, et cetera

In the absence of thyroxine the metabolism of the cells, and the body, drops to a lower level where it remains until thyroxine is again supplied A variety of physiologic activi ties are involved in this fundamental effect on the metab olism of the cells Means[1] has listed them as follows

1 Calorigenic action
2 Action upon the growth, maturation, and differentia tion of tissue
3 Action upon the distribution of body water, salts, and colloids
4 Action upon carbohydrate metabolism
5 Action upon the nervous system
6 Action upon the muscular system
7 Action upon the circulatory system
8 Action upon other endocrines

As is the case with the vitamins the action of thyroxine though universal in respect to the cells is expressed by its particular effect on certain functions and in human disease the clinical manifestations are in general confined to those organs or functions It is this which gives the characteristic pattern to the various deficiency diseases Unlike most of the vitamins, however, thyroxine taken in excess has pharmaco logic and toxic properties which are clearly recognized Iodine, also, in amounts greater than those required for the

physiologic functions, as stated above, possesses toxic and pharmacologic actions which are familiar to all physicians

PATHOLOGY AND PATHOGENESIS

Two pathologic conditions result from a lack of iodine—goiter and a hypofunction of the thyroid gland (hypothyroidism) Both of these may be present or either may occur without the other, but when hypothyroidism is due to iodine deficiency it is usually accompanied by a goiter The two should be sharply differentiated because of their totally different effects on the body Goiter can be present without interference in any body function except such as may be caused by a localized tumor Hypothyroidism on the contrary affects many body functions and affects the growth and activity of all tissues of the body In its most severe form it results in greater or lesser grades of cretinism From a practical point of view this is always of the endemic variety It is possible that an individual child might, through some strange circumstance, remain on such a low iodine intake that a *sporadic* cretinism based on iodine deficiency would develop This is extremely infrequent and sporadic cretins are almost always *athyroidic* for some other and usually unknown reason In a similar way an adult could conceivably receive so little iodine over so long a period that hypothyroidism would develop Adult myxedema is however almost always due to other causes The pathological expression of an inadequacy of iodine, then, is *simple goiter** and if continued over generations *endemic cretinism* The latter is seen only in goitrous regions and is probably rarely seen

* Following Marine and Means we have called the goiter caused by a deficiency of iodine simple goiter The objections to the various terms in use have been discussed by Means His suggestion of iodine-deficiency goiter or better iodine-deficiency disease would be particularly apropos for this book but as he adds the term is clumsy and not in common use

in this country, though mild grades of hypofunction may b
encountered

Simple goiter is an enlargement of the thyroid glan
(hyperplasia and hypertrophy) resulting from the increase
work thrown on it by the effort to extract iodine and manu
facture thyroxine as the available supply of iodine falls I
is important to remember that this enlargement, as previ
ously stated, does not necessarily mean a deficient secretio
of the hormone As Means has said, 'the factory may b
working under difficulty, the supply of raw material de
creased, overwork and additional machinery may be needed
but until the supply of iodine is used up hormone in essen
tially normal amounts is furnished ' The effect of th
effort on the thyroid itself is observable in the pathologi
changes in the gland When the supply of raw materia
(iodine) finally becomes absolutely inadequate the suppl
of hormone to the tissues is insufficient Then the pathologi
changes as well as the symptoms of hypothyroidism appear
giving in the more severe cases the well known patholog
and clinical picture of cretinism Such a failure of the hor
mone rarely occurs except on a basis of iodine insufficienc
over several generations Children born of goitrous mothers,
in whom the goiter is due to iodine lack, will exhibit a
greater tendency to goiter, their children will in turn show
the tendency to a still greater degree and this process will
continue until cretinism results Cretins may or may not pre
sent goiters

Although it is a deficiency of iodine which causes the con
ditions just described, the deficiency may be absolute or rela
tive and secondary factors may be involved An absolute de
ficiency is the principal factor in goitrous regions and may,
and often does, produce goiter without the intervention of
secondary causes Relative deficiency is more important in
regions of a fair to good iodine supply, and in individual

cases may cause disease even when the iodine supply of the region is entirely adequate

The secondary factors leading to relative insufficiency are increased requirement and failure to absorb or utilize available iodine Increased demand occurs from many causes, normal or physiologic and abnormal or pathologic Body size, growth puberty, pregnancy and probably menstruation are physiologic factors which affect the need for iodine With a liberal supply extra demands are met without the occurrence of pathologic changes But a supply which is just sufficient under ordinary conditions, may be inadequate when extra demands are made The influence of age, growth, puberty, pregnancy and menstruation are the reasons for a greater incidence of iodine deficiency in children and in females Pathologic causes for increased requirements are perhaps infections and pollution of water or food supplies, but conclusive evidence of such an effect has not been presented for either of these latter factors

Another way in which a relative shortage of iodine might be produced is by interference with the oxidative processes in the tissues causing a demand on the thyroid for an additional supply of the hormone (thyroxine) this in turn leading to an increased need for iodine and a relative deficiency of the latter in cases in which the supply is minimal or borderline This has been demonstrated by producing goiters in animals by feeding them certain vegetables such as cabbage and cauliflower The organic cyanide in these vegetables depresses tissue oxidation and causes a demand for more thyroxine Such goiters can be prevented by giving iodine and the goitrogenic action of these foods is counteracted by some unknown substances found in other plants such as green grass and alfalfa or even to some extent in cabbage itself Just how great an influence the effect of such goitrogenic foods exerts in humans is unknown but it is

conceivable that under certain conditions of diet and iodin
supply it might be significant

A relative insufficiency of iodine due to difficulty in ab
sorption and utilization is probably very infrequent thoug
infections might conceivably produce this effect Iodine i
very easily absorbed Similarly, unless the thyroid glan
were rather severely diseased it is improbable that difficult
in utilization would occur There is a large margin of safet
in the functioning mass of the gland Little is known of a
possible deficiency of the other substances combined wit
iodine to form the completed hormone but such a deficienc
is unlikely An absolute deficiency of iodine, then, or a
relative deficiency due to a borderline supply and a require
ment increased by physiologic demands, remain the mos
frequent causes of simple goiter

The shortage of iodine required to produce pathologi
changes has been accurately determined pathologically
When the concentration of iodine in the thyroid gland fall
below 0 1 per cent (dry weight) hyperplasia occurs Suc
a measure of deficiency is of no practical use clinically I
merely expresses the level of reserve hormone which th
thyroid is committed to maintain When the reserve fall
below this level more hormone must be manufactured and
stored, and if the supply of iodine is too small to allow i
to be extracted from the blood by the normal gland, hyper
trophy and hyperplasia develop to further the process The
result is a goiter

The pathology of goiter due to iodine deficiency varies
both grossly and microscopically with the various stages of
the disease and the effect of secondary changes In the begin
ning of hypertrophy and hyperplasia the gland enlarges, be
comes softer and more vascular In the cut surface it appears
more meaty and the colloid is decreased Microscopically
the epithelium changes from low cuboidal and cuboidal to
high cuboidal or even columnar There is a decrease in

stored colloid, increased vascularity and a decrease in iodine content. With this the follicles become infolded with papillary projections into the lumen and are no longer round or oval but wrinkled. The cells show increased mitosis. There is some doubt whether actually new follicles are formed.

This is the stage of active hyperplasia and hypertrophy which occurs as a result of insufficient iodine. It is essentially similar to that seen in the hyperplasia of pregnancy or puberty and perhaps in other conditions of increased physiologic demand. It also constitutes the first stage in the thyroid cycle of Marine.

According to Marine[2] the next stage is either atrophy or *involution. If the deficiency of iodine* persists the over worked cells eventually atrophy and die. In such cases, according to Marine the most striking changes are seen in the epithelium and stroma. In the first stages the picture is that of an extreme degree of active hyperplasia with only an occasional pyknotic or atypical cell mass. In the more advanced stages the cells lose their uniform arrangement in the follicle wall. Desquamation and disintegration of the cells may be seen in the same follicle with mitotic figures and hypertrophied cells. Nuclei of the affected cells show great irregularity, sometimes they are enlarged and hyperchromatic, sometimes small and pyknotic, but always variable in size and staining intensity. The colloid is usually greatly reduced, although occasionally dense masses of colloid may appear to be imbedded in the stroma with little remaining evidence of the original follicular epithelium. As the atrophic process continues the follicle becomes reduced in size by cell death and advancing sclerosis, until the remaining epithelial cells appear as compressed nests of distorted cell masses. The stroma is always relatively increased and in some instances seems to be absolutely increased.

The alternative to this and the process which usually occurs (in this country at least) is involution. This is because

an adequate supply of iodine usually becomes available before very great atrophy can ensue Involution is a reversal of the changes which occur in hypertrophy and hyperplasia The gland becomes redder and firmer The blood supply diminishes Microscopically colloid again fills the follicles, even overfills them and distends them The epithelium shrinks to cuboidal or low cuboidal, the stroma becomes less prominent The iodine content of the gland rises

Some difference of opinion exists as to whether the gland ever returns to an entirely normal state after such an experience Marine believes not and thinks that the colloid phase is only the closest approach to normal such a gland can attain Certainly this is true in some cases and the enlarged gland with distended follicles filled or overfilled with colloid constitutes what is commonly called the colloid goiter

Such a result may, however, merely represent an incomplete involution Clinical experience teaches us that many hypertrophic and presumably hyperplastic glands, due apparently to absolute or relative iodine deficiency, may resolve completely (disappear) This would indicate that such is the normal course of involution, and failure to do so would indicate an arrested involution Also, as Means[1] suggests, colloid stored too long under these conditions may undergo changes which render it incapable of resorption This may be the case in old, large, edemic, colloid goiters

Having involuted and returned to normal the gland may again encounter iodine deficiency, relative or absolute, again become hypertrophic and hyperplastic, finally to involute once more According to Marine this cycle may be, and often is, repeated several times With each cycle the reversion to a normal state becomes less likely If the gland fails to return completely to normal the first time, or at subsequent periods, each period of partial involution and colloid deposit leaves the goiter a little larger Furthermore, during involution, particularly incomplete involution, localized

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In summary the pathologic changes in the thyroid due to iodine lack are first a hypertrophy and hyperplasia of the secreting cells, loss of colloid and increase in vascularity associated with a relatively soft enlargement of the gland (goiter) without thyrotoxicosis This is without effect on the body tissues generally unless the iodine shortage is so severe and long continued that it leads to extensive exhaustion atrophy of the secretory tissue This rarely occurs except in individuals who are the unfortunate possessors of thyroid glands subnormal from the start as a result of several genera tions of mothers who lacked sufficient iodine With an ade quate supply of iodine hypertrophy and hyperplasia disap pear, colloid appears in the follicles, and vascularity diminishes This change is accompanied by an increased firmness of the goiter In favorable cases, and these may be the majority except in more severely goitrous areas, involu tion then is completed the gland diminishes in size an the goiter disappears In other cases involution is arreste in the colloid stage, and the simple or colloid goiter persis

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INCIDENCE AND EPIDEMIOLOGY

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with or without additional features such as nodules Successive cycles increase the likelihood that resolution will be arrested in the colloid stage, similar occurrences in successive generations make likely the occurrence of cretinism

The pathologic changes in cretinism differ from those occurring in simple goiter in that there are widespread abnormalities throughout the body as well as more severe damage to the thyroid gland Locally the thyroid gland may show changes in size varying from complete or nearly complete absence to large goiters which microscopically show the changes seen in the milder simple goiter In the cretin, however, despite the presence of a goiter, there is at least a partial failure of function, a failure sufficient to cause general body effects Usually the functional efficiency of the thyroid gland is very poor The fact that it does have some slight functional capacity explains the varying degree of cretinism which may occur in endemic cretinism In this respect endemic cretins differ from the sporadic cretin in whom the loss of function is almost always complete (*athyreosis totalis*) Juvenile myxedema occupies somewhat of a midposition It may be defined, as Means[1] has done, as a state of athyreosis acquired by a previously normal child prior to the attainment of puberty and full growth As such it is rarely, if ever, due to iodine deficiency and is similar to adult myxedema

In the thyroid gland the changes are essentially those of a replacement of normal structures by fibrous tissue in which there may be scattered follicles some of which may contain colloid In the less advanced cases there is an atrophy of the secreting tissue with an infiltration of lymphoid and plasma cells about the follicles which preserve a shadow of the former outline of the follicle The gland may be much smaller than normal, in fact in some cases practically non existent, but in some there is a considerable goiter This is, however, composed almost entirely of fibrous tissue

The general body changes in cretinism are dominated by retardations in development These include delayed union of the epiphyses imperfect and delayed ossification of the skeleton, faulty and delayed dentition and incomplete de velopment of the brain Hypertrophy of the anterior lobe of the pituitary is not uncommon and is a significant finding in view of the recent discovery of a thyrotropic hormone secreted by this organ This suggests a hyperfunction of the anterior pituitary in an attempt to stimulate a deficient thyroid Hertz and Castler[3] have shown that in myxedema there is an excess of anterior pituitary thyrotropic hormone in the blood and urine

INCIDENCE AND EPIDEMIOLOGY

The incidence of iodine deficiency with its sequelae, goiter and cretinism varies geographically with variation in the iodine content of the soil In some regions, parts of Switzerland for example, the content is so low that iodine deficiency is or was, practically universal In other regions such as the sea coast the supply of iodine is so great that goiter occurs only rarely and sporadically in those indi viduals who develop it for some reason peculiar to them selves Although regions of endemic goiter are apt to be mountainous ones they can also be found in the plains, as in the Great Lakes region of the United States

In this country there are no districts with as great a deficiency of iodine and hence as goitrous as some in other parts of the world There are however areas in which there is a significant shortage of iodine, and a considerable degree of goitrousness The latter has been greatly re duced by prophylactic treatment The areas include the northwestern group of states and the Great Lakes region especially Michigan Wisconsin and Colorado Olesen finds the areas of highest incidence to have 10 to 27 goiters per

1000 individuals and the lowest to have 1 per 1000 Small local areas may show a much higher incidence

Goiters due to a deficiency of iodine are more frequent as age increases through puberty It is also more common in girls than in boys with the preponderance becoming less marked as the region becomes more deficient in iodine until in very deficient areas the disease occurs with about equal frequency The greater incidence in girls in regions where the deficiency is less severe may be related to the antagonistic action of certain sex hormones, these girls may require a somewhat greater supply of iodine than do boys

SYMPTOMS AND SIGNS

The only clinical expression of simple iodine deficiency is a goiter (simple goiter) As previously explained, in the absence of cretinism there is no interference with general body functions, and the only symptoms and signs are those associated with the goiter In the cases developing early in life the child will present, at about five or six years of age, a fulness in the neck, palpable first, then visible The enlargement is smooth, soft, uniform, symmetrical, without thrill, bruit, or tenderness Associated with the enlargement there may be a sense of fulness in the neck but often the goiter is first noticed when it becomes visible to the family or friends The child may appear somewhat delicate but otherwise is essentially normal In a goitrous district the number of children developing a goiter and the size of the goiter increases with age so that not only do more children have a goiter but there are more large goiters in older children The incidence among girls is greater than among boys In regions without endemic goiter the number is, of course, much smaller and the goiter is due to some peculiar individual circumstances causing an iodine deficiency, not a diminished supply common to all Similarly, in areas of

endemic goiter the failure of some to develop a goiter is dependent on individual circumstances which avoid a short age of iodine or make it less severe (relative or absolute)

With advancing age (beyond puberty) some of the goiters will disappear and some will become smaller This is much more frequent in boys Girls are apt to show an increasing frequency and an increase in the size of the goiters to an older age (17 18) as well as much less tendency for the goiter to disappear In the majority of girls a goiter persists and even increases slowly in size Pregnancies have an important influence on the size of the goiter As the goiter becomes smaller it often becomes firmer It may even become larger as it gets firmer This is the colloid storage phase (colloid goiter)

In girls and women temporary enlargement of the thyroid may occur with menstruation and it is probable that other factors such as emotional stress, infections and other dis eases may cause recurring temporary enlargements which recede to the original state It is very important to recognize that the temporary slight enlargements which may appear under these circumstances do not represent a true pathologic enlargement Some of these enlargements may be due to a temporary relative shortage of iodine and hence are true iodine deficiency goiters in the strict sense Others may have no relation to deficiency The importance of this lies in respect to drastic treatment (surgery) in these cases Serious mistakes may be made if the attitude is taken that a lump in the neck means goiter and goiter means surgery

Aside from appearance and perhaps a slight sense of ful ness in the neck, often exaggerated by nervous young girls and women these goiters may cause no symptoms Pressure effects may be caused, especially by those of large size This is unusual, however, in sporadic cases in young persons or even in regions of endemic goiter in this country

Women are apt to show an increase in the size of the

goiter when they become pregnant (The goiter may first appear during pregnancy) The goiter tends to become smaller after delivery but with each pregnancy it enlarges, never returning to the original size so that with each pregnancy there is an increase in size It would appear that this may occur even with a supply of iodine which would normally be adequate, the defect in the gland already present making it so difficult to function that a low normal supply becomes insufficient, *a relative deficiency* Such enlargements may be prevented by an excess supply of iodine Few such women exhibit general symptoms of thyroid deficiency during pregnancy or afterwards but some may exhibit mild hypothyroid symptoms, a true thyroid deficiency resulting from changes in the gland primarily due to iodine deficiency Children of such women may have a somewhat greater tendency to the development of goiter than children born of normal mothers and the process, if continued through a sufficient number of generations results, of course, in cretinism

During the middle and later years there is a considerable tendency for the goiter to become nodular Such goiters present the complications described under the section on pathology and the development of nodules and the generally greater size of the goiter in these persons causes a more frequent occurrence of pressure symptoms and signs These include pressure on the trachea, with deviation and often compression, sometimes so severe as to interfere with respiration, or even to cause complete obstruction Associated with this there may be cough, difficulty in swallowing, venous obstruction, edema of the face, pressure on nerve trunks, and in some cases even death Nodules may become cystic and then are subject to hemorrhage within, resulting in pain and swelling Sometimes infection causes suppuration There is also a distinct tendency to malignancy

In regions of more severe endemics, and occasionally in

sporadic cases, more severe signs and symptoms are encountered The goiters are much larger and huge ones are found Goiters appear earlier in life, even in infants and evidence of hypothyroidism appears This means cretinism, which, as already explained, will vary in severity and is usually accompanied by goiter in contrast to the lack of a thyroid gland in the ordinary sporadic cretin Such endemic cretins are born with goiters and are the end result of a succession of increasingly goitrous iodine deficient, and, finally, hypothyroid ancestors (mothers) Most are imbeciles from birth and respond poorly if at all to thyroid extract particularly if it is not begun early in life As already stated such endemic cretinism is not found in this country

DIAGNOSIS

In the past the diagnosis of iodine deficiency was in effect the diagnosis of simple goiter and the fact that iodine deficiency is present before the goiter was neglected Thus the opportunity of diagnosing the deficiency before pathologic changes occurred and hence the opportunity of preventing the latter was missed The diagnosis is, of course, simple and consists of the recognition of a deficient intake For years this has been easy in areas of endemic goiter The lack of iodine has been a matter of common knowledge and prophylactic treatment has been successfully applied When that has not been done the failure has been due to simple neglect However, the diagnosis is not so easy in sporadic cases or in regions where the deficiency is less common It may be suspected from a knowledge of the iodine supply of the region and a knowledge of the patient s diet In such cases contributory factors become very important Unfortunately, these are not always evident Some, however, such as pregnancy, are easily observed In sporadic cases then and in neglected cases in areas of endemic goiter, the diagnosis of

iodine deficiency is usually not made until the goiter appears The diagnosis of simple goiter depends first on detection of a goiter and then on the differentiation of it from other goiters First it is necessary to define the degree of enlargement of the gland that constitutes a goiter This may be taken as an enlargement which causes the gland to become palpable The normal thyroid gland is generally considered to be non palpable although those who are accustomed to examining carefully for goiter often feel a gland which is slightly palpable, especially in the isthmus This is not considered pathologic but anything more can be considered a goiter

In young people with simple goiter the gland is diffusely and symmetrically enlarged It is soft, the borders of the lobes cannot be easily distinguished and it moves readily on swallowing Bruit and thrills are lacking and there are usually no nodules In older subjects the gland as a rule is more firm and is apt to be nodular In this country large goiters are uncommon

The principal differential diagnosis is between simple goiter and so called toxic goiter (Graves disease, exophthalmic goiter, goiter with hyperthyroidism, et cetera) Signs and symptoms of toxicity, including particularly an elevated basal metabolic rate, are incompatible with simple goiter though toxic goiter can and does develop in patients with pre existing simple goiter Malignancy, too, may occur in simple goiter and presents the difficulty in diagnosis that characterizes malignant neoplasms of the thyroid Consistency, evidence of attachment to neighboring structures, immobility of the gland, evidence of metastases, et cetera, are some of the common findings in malignancy Inflammation, thyroiditis, also may cause a goiter (enlargement) but is associated with local evidence of infection and inflammation which are sufficient to distinguish it from simple goiter Inflammation may however, develop in a simple goiter

Hypofunction can, of course, occur with simple goiter As cretinism this is well recognized and has been discussed in previous sections The exact significance of mild grades of hypofunction, manifested principally by a lowered basal metabolic rate is less clear For the present and for the purposes of this book they may be best considered as disease of the thyroid other than simple goiter This is principally because they do not ordinarily respond to iodine but require thyroid extract for treatment

TREATMENT

The preventive or prophylactic treatment of goiter due to iodine deficiency is highly successful, treatment once the goiter has developed is not Therefore, to a greater degree than with some other deficiency diseases prophylaxis is more important than curative treatment Iodine in almost any form can be used and if given in the amounts needed one preparation offers few, if any, advantages over another except in relation to availability, palatability and cost

Prevention As previously stated the human requirement of iodine is around 0 02 to 0 075 mg (20 to 75 gamma) per day Actually this is the minimum requirement and the smaller amount, 0 02 mg is probably not entirely sufficient This amount can be readily obtained in the food and drinking water in regions where the soil contains sufficient iodine and if sea food is eaten Even in regions where the soil and water are less rich in iodine, a proper diet which includes a fair proportion of leafy vegetables will provide sufficient iodine Only if the diet is grossly abnormal is a shortage likely

When, however, the concentration of iodine in the soil (plants) and water of a region is low and the region is so large that food supplies from other places are not important in the dietary of the population, specific prevention is indi

cated and necessary This is easy to determine and in practice physicians should inform themselves of the status of their community in this respect and be prepared to provide preventive treatment in case mass prevention is not provided for by public measures

Mass prevention at the present time is almost confined to the use of iodized salt Iodinization of the water supply has had only a very limited application Iodized salt can also be used for families or even for individuals The usual concentration is 1 100,000 in this country, which on the basis of 10 Gm of salt daily will provide 100 gamma The Swiss use a much lower concentration fearing the induction of thyrotoxicosis in nodular goiters (Jod Basedow) I believe this fear is groundless Iodized salt, then, is an effective means of prophylaxis against simple goiter on a family basis, and in areas of known edemic goiter its use should be made general, through public health measures if necessary

Individual prevention, as already indicated, can be accomplished by the use of iodized salt but it is also often affected by the adminstration of iodine in other ways For this purpose iodine has been administered in many forms, sodium iodide, syrup of hydriodic acid, tincture of iodine, et cetera, in varying doses and for various periods For general use Means recommendation of one drop of Liquor Iodi Compositus, U S P , per week will be very satisfactory This provides about 243 gamma per day, and in the case of children particularly it may be given over a period of three or four weeks, spring and fall Other forms of iodine may be used in comparable doses Larger amounts, 10 mg per day, have been recommended and are not harmful, but in the past have been feared because of possible danger It is unlikely that there is any danger in these amounts but they are larger than necessary

In addition to the use of iodine, diet and general hygiene should be considered in the prevention of goiter With

regard to diet there is the possible benefit to be gained from the inclusion of a proper amount of fresh vegetables An ordinary or even excessive consumption of foods will not protect against goiter in regions where there is any con siderable iodine deficiency Iodine will be necessary there but diet will help in borderline areas However, one must also consider the possible goitrogenic effect of an excess of such vegetables as cabbage If such vegetables exert such an effect it is possible that in large amounts they might render a bor derline supply of iodine inadequate and make prophylaxis necessary Whether polluted water is a factor in producing goiter is uncertain but in any event a supply of pure water, adequate nutrition, et cetera may have a favorable influence on the incidence of simple goiter by their effect on general health

Protection Besides the general prevention of goiter and iodine deficiency two groups of persons require special *pro tective* treatment These are pregnant women and pubescent children (especially girls) This protection will be needed most in regions where the iodine supply is minimal or bor derline as regards adequacy In frankly deficient areas gen eral prophylactic treatment protects or should protect them In regions where the iodine supply is liberal protection may not be required In the borderline areas, however, deficiency is apt to develop under the stress of increased demand and even in presumably safe regions the possibility of individual deficiency justifies that all pregnant women receive supple ments of iodine

For pregnant women the dose already mentioned for pro tection, about 0 25 mg per day, represented by one drop of Liquor Iodi Compositus U S P, (Lugol s solution) a week during the last half of pregnancy or a comparable amount of other preparations of iodine will be adequate The same amount (one drop) may be given three or four days a week for one week out of each month for the same

period For children at puberty a drop of Liquor Iodi Co positus, U S P, (Lugol s solution) two or three times week for two or three weeks in the spring and fall w provide sufficient protection

Curative Treatment Once a deficiency of iodine h caused a goiter, treatment is much less successful Althou an adequate intake of iodine relieves the shortage, the resu of the deficiency (the goiter) is apt to persist unless remov surgically

Small goiters may be treated with iodine with a fa chance that the goiter will be reduced in size and will occ sionally practically disappear in young people For this pu pose iodine should be given in somewhat larger doses tha are generally used for prevention One to five drops Liquor Iodi Compositus, U S P a day for a period of tw to four weeks, repeated at intervals of three or four wee over six months or a year may be tried Similar treatmen for a two to four weeks' period each spring and fall may b employed as in the prophylaxis of goiter Sodium iodide i doses of 0 001 to 0 01 Gm (1/60 to 1/6 gr) may be use in place of the compound solution, as may equivalen amounts of tincture of iodine, potassium iodide, syrup o hydriodic acid, or any other iodine compound The objec is to administer one to 10 mg of iodine daily, the form i which it is given makes little difference so long as th amount of iodine is provided

In some cases such treatment with iodine will be followed by a decrease in the goiter, sometimes by its disappearance Occasionally the gland will become somewhat larger an more tense or firm This may cause the patient or her famil some concern but is only rarely of any serious significance In all but a rare case it may be interpreted as the distension of the hyperplastic gland with colloid formed as the result of an adequate supply of iodine The fact that such cases of enlargement and increased firmness are uncommon is be

cause most glands have become filled with colloid before they are seen by the physician and iodine has little effect of any kind Rarely, iodine given for what appears to be a simple goiter will actually be given to a patient who is developing thyrotoxicosis In such a case firmness and in crease in size will develop Such a mistake may occur because the toxic change is so early as to defy detection Such cases are uncommon

Most authorities advise against giving iodine to patients with nodules in the goiter because of the supposed danger of inducing thyrotoxicosis I have already stated that I be lieve this danger is non existent but I would advise against iodine in these cases for other reasons Nodular goiters respond poorly, if at all to iodine They are usually found in older persons with larger goiters circumstances which ordinarily indicate a different form of treatment (surgery) Sometimes the nodules are accentuated in appearance by treatment with iodine It is not always possible to feel nodules, especially in rather large, firm goiters, and the nodules sometimes become apparent only when the other parts of the gland have involuted following treatment with iodine In the patient s eyes this may constitute a somewhat unfavorable result of treatment from the point of appear ance and emphasizes the need for careful examination be fore treatment as well as the infrequent use of iodine in older men and women in whom nodules are more common

Little trouble from iodine is to be expected with doses of the size recommended but it may occur in susceptible cases Occasionally iodine will cause a drop in the basal metabolic rate of persons with simple goiter This drop is only temporary and has no untoward effect

Thyroid extract has been recommended and used in the treatment of simple goiter, presumably in the belief that the hyperplasia and hypertrophy caused by the lack of iodine can be relieved by an exogenous supply of thyroid hormone

While theoretically such treatment might be effective before hyperplasia and hypertrophy (the goiter) have developed it can be expected to have but little influence once the goiter has appeared I have observed occasionally a patient in whom treatment with thyroid extract seemed effective in reducing a goiter and it may be tried cautiously in se lected cases Thyroid extract contains iodine, of course, and its beneficial effects may be due to the iodine rather than to the hormone itself It is too dangerous to be used as a general prophylactic against goiter But, it should be used when there are associated symptoms of hypothyroidism and a lowered basal metabolic rate Such cases are not com mon except in areas of endemic goiter

Recently, thyrotrophic hormone (pituitary), which stimu lates thyroid function and leads to a loss of colloid from the gland, has been suggested for the treatment of simple goiter So far too few cases have been reported to warrant recommendation for general use It may be tried cautiously Because the effect of thyrotrophic hormone gradually dimin ishes and disappears it is unlikely that careful administration would result in a dangerous overstimulation of the thyroid gland

Surgical removal is the only satisfactory treatment for the larger goiters and may be necessary, even urgently needed, in cases where the goiter causes pressure in the trachea or other organs and tissues This is especially true of the so called intrathoracic goiter *Except, however, in those cases* in which pressure symptoms exist, surgery should be de layed until adulthood Most small goiters that are barely visible and palpable should not be removed Childhood and puberty are a time of intermittent stress and strain and premature removal of an enlarged thyroid may be followed by an uglier and more disfiguring hypertrophy of the re maining fragments or even the development of a hypo thyroidism With maturity the gland is less subject to un

usual stimulation (except for pregnancy which should call for special protection) It has become stabilized and may be removed with more satisfactory results Also, at this period and with such goiters, removal may be good prophylactic surgery against cancer and perhaps against thyrotoxicosis which may be more apt to occur in persons with such goiters Surgical removal also prevents such complications as hemorrhage into cysts

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13

Other Possible Nutritional Deficiencies

IN ADDITION to the essential food factors discussed in previous chapters there are a number of other nutrient substances a deficiency of which causes disease or is suspected of causing disease For some of them there are as yet no known clinical manifestations and this is particularly true of some vitamins or vitamin like substances which have been shown to be essential for other animals or organisms but not as yet for man These substances are briefly discussed here because speculation or experimentation from time to time raises a question of their relation to human nutrition and health, and it is felt that a brief statement of the current clinical status of these substances is desirable It seems desirable also that the practicing physician be made aware of the direction of research in this field

These substances may be divided into three classes as fol lows (1) Vitamins or vitamin like substances which have been discovered and shown to be necessary in the nutrition of other organisms but whose relation to human nutrition and health is unknown or uncertain (2) The so called trace elements, minerals which may be essential to humans but if so are required in such small amounts that a deficiency is difficult to conceive in view of their ubiquitous occurrence in adequate amounts in food or water, (3) Substances which are necessary and are needed in relatively large amounts by

the body but are so liberally supplied by the diet that clini cal deficiencies are rarely, if ever, encountered

VITAMINS AND VITAMIN LIKE SUBSTANCES

This group includes pantothenic acid pyridoxine (B_6), para aminobenzoic acid, inositol (all members of the B complex), biotin (vitamin H), vitamin P, the unidentified vitamins, and choline

Pantothenic acid, also known as the filtrate factor, anti dermatosis vitamin, etc, is a peptide of β alanine and is found widely distributed in animal and vegetable tissues, especially in the viscera of animals It is believed to be an essential requirement of all living matter but various organisms differ in their ability to synthesize it It is found in the blood and urine of man, and having been found essential to higher animals (dogs, etc) has been assumed to be necessary for man also Its function in man is unknown, as are the pathology and pathologic physiology Rats develop hemor rhages and atrophy and necrosis of the adrenals when given pantothenic deficient diets and the diet also causes depig mentation of the fur (nutritional achromotrichia) in some animals No specific tests for the manifestations of a defi ciency (if any) in man exist The concentration in the blood and urine can be determined by microbiologic methods and tests of blood levels and urinary excretion may be helpful in clinical studies Normally, the concentration in the blood is said to be around 0 2 to 0 3 gamma per cc Decreased concentration and diminished excretion have been reported in subjects with pellagra and beriberi Re quirements for man are unknown and no toxic manifes tations have been reported It is available in pure form and dosage can be expressed by weight (mg) No standards have been established

Pyridoxin (B_6 also known as the anti acrodynia rat fac tor, rat antidermatitis factor and by other names) is a

pyridine derivative It is widely distributed in nature and is especially abundant in yeast and rice polishings Its physiologic action in man is unknown, and its essentiality though suspected has not been proven Similarly, the manifestations (if any) of its deficiency are not clearly recognized Its use in patients with pellagra has been reported to result in additional improvement in such symptoms as nervousness, dispositional changes, and weakness after adequate treatment had been given with niacin, thiamin, and riboflavin It has also been reported to cure glossitis and cheilosis after riboflavin had failed In animals its deficiency has been associated with peripheral nerve lesions resembling those of thiamin deficiency, epileptiform fits, muscle degenerations, and anemia On this basis it has been used clinically in certain myopathies, anemias, and epilepsy, but its value has not been established and critical analysis as yet fails to disclose a significant action Deficiency may be suspected on the basis of the relationships above The presence and concentration can be determined by a variety of chemical and biologic methods but the only one in any clinical use is a microbiologic test (bacterial) Excretion in the urine can be determined but its significance is not clear and no established standards are available The possible daily requirements for man are unknown and no significant toxicity has been reported It has been isolated and synthesized and is available in pure form with dosage expressed by weight Because they are components of the B complex, it and pantothenic acid are often included in compound pharmaceutical vitamin preparations

Para-amino-benzoic acid, known also as the anti gray hair factor, is a substance of obvious chemical composition which has been shown to be an essential dietary factor (chromotrichic) for the rat and (growth promoting) for the chick It is also an essential for the growth of certain bacteria, notably the *Brucella* and *Streptococcus hemolyticus* It was its anti sulfanilamide action which led to its discovery as a

vitamin. It is a constituent of yeast. Little or nothing is known of its physiologic function in man though its effect in inhibiting the action of the sulfonamides suggests that it may be related to enzyme activity and under abnormal conditions, such as exposure to toxins, may play a role. This is different, however, from a normal nutritive action. In the black or piebald rat a deficiency causes a graying of the fur (nutritional achromotrichia) and its administration has been said to halt graying in man and to restore normal color. This has not been confirmed. It has been used in asthma in man on the basis of its action in protecting adrenalin against oxidative destruction. Actually, however, its need, daily requirements and manifestations of a deficiency, if any, are unknown. It is available in chemically pure form and is essentially non-toxic.

Inositol (also known as Bios I, the mouse anti-alopecia factor, and by other names), is another substance which has a well-nigh universal distribution in plant and animal tissues. Some fruits, cereal grains, yeast, molds, and bacteria are good sources. It may be synthesized by some animal organisms. It exists in many forms but the optically inactive form (i-inositol or meso-inositol) is the only one of nutritive importance. In animals a lipotropic action has been described but nothing of its possible function in man has been established though an effect on motility of the gut has been described. Daily requirements and manifestations of deficiency are unknown. It can be determined by microbiologic methods but these are not employed clinically. It is available in pure form and apparently is not toxic.

Biotin (also Bios II, vitamin H, anti-egg-white injury factor, factor W, etc.) is a growth factor for certain bacteria, yeast and higher plants and is a required factor for some animals. An experimental deficiency has been produced in man.[4] Biotin is widely distributed in plants and is found in smaller amounts in animal tissues. Biotin has been thought to have a part in fat metabolism in animals but its known

action is combating egg white injury. Raw egg white contains a protein, "avidin," which binds biotin and makes it unavailable to the organism. Thus, feeding egg white induces biotin deficiency, supplements of biotin prevent or cure the deficiency. Animals (rats and chicks) develop a dermatitis and other lesions. Experimentally, the administration of dried egg white in amounts equal to 30 per cent of the calories of the diet has produced a peculiar ashy pallor of the skin and mucous membranes with dryness and desquamation, lassitude and somnolence, muscle pains, precordial distress, and anorexia. All symptoms were relieved by the parenteral administration of biotin. No naturally occurring syndrome of biotin deficiency is recognized,* and in view of the severe conditions required for experimental deficiency a spontaneous deficiency is probably unlikely and infrequent. In this regard biotin can be grouped with certain other newly recognized and possibly essential food factors of which there are no ordinary nutritional deficiencies but which may become deficient with exposure to toxic agents, under conditions of abnormal metabolism, etc. Unconfirmed therapeutic effects with biotin have been reported in various skin diseases. It has been prepared in pure form but is ordinarily available as a concentrate. Besides the unit of weight (gamma) amounts are expressed in "Rat units" and "Saccharomycetes units." Determinations can be made by biological methods, of which the most useful are the microbiologic.

Vitamin P (Citrin). Besides vitamin C another vitamin having to do with the capillaries and their permeability and hence hemorrhagic diatheses has been postulated, namely, vitamin P. Vitamin P is not known in pure form, but is found in concentrates containing eriodictin and hesperidin, glucosides of eriodictyol. The supposed vitamin P is found in fruits and vegetables, especially in citrus fruits, but

* Williams reports a possible case in New England Jour. Med. 228: 247, 1943.

has not been demonstrated in animal tissues Vitamin P is said to be concerned with the maintenance of capillary integrity, and a deficiency is said to result in increased capillary fragility, permeability, and hemorrhages Its action is said to be distinct from that of ascorbic acid (vitamin C) but may be associated with it A deficiency in man is said to be represented by a hemorrhagic state (purpura) which does not respond to vitamin C but does to vitamin P However, the published reports have not been very convincing and numerous hemorrhagic states not responding to vitamin C have also failed to be cured by vitamin P No test for the deficiency other than a therapeutic trial exists, and no other method for its determination is available No standards have been devised, and the concentrates are not available for general clinical use

Non-identified Vitamins In addition to the vitamins which have been discussed there are a number of substances which may or may not be vitamins Some are hypothetical, supposititious substances whose existence is suspected because of experimental work suggesting as yet unrecognized essential factors Others are known substances, such as adenylic acid, which are suspected of having a vitamin function which as yet has not been demonstrated Many are undoubtedly identical with vitamins already identified, others may be essential for some organism or another, some undoubtedly do not exist

PROTECTIVE FOODS

Besides the vitamins there are certain substances which are necessary for growth and nutrition and which man cannot synthesize Unlike the vitamins they also furnish energy or enter into the structure of the body To these substances Rosenberg has given the name "vitagen In a sense carbohydrate itself might be called a vitagen but the essential amino acids, discussed in the chapter on proteins, are a better example Others are the *fats* and *choline*

Fats. Fats are not only good sources of energy, but they are also carriers of certain vitamins, such as A, D, K, and E. In addition to this, however, there is some evidence that certain unsaturated fatty acids are essential in small amounts for the growth and health of some animals. These may be linoleic and linolenic acid which will at least relieve the symptoms thought to be caused by a deficiency of fatty acids in rats. It may also be that these fatty acids are converted in the body into arachidonic acid—a fatty acid also effective in relieving fat deficiency in rats. However, fatty acid deficiency probably plays no known part in clinical medicine.

Choline. Choline is a supplier of the transferable methyl group, resembling in this function the amino acid methionine which it spares. In the absence of choline, methionine may be insufficient for its normal function because it is diverted to furnishing its labile methyl group. Choline is involved in cell structure through the phospho-lipids and in neuromuscular function as acetylcholine, also thought to be concerned with fat metabolism. Its deficiency may be reflected in the occurrence of a fatty liver. In animals a number of other pathologic changes resulting from choline deficiency have been described, such as cirrhosis of the liver. However, ordinary nutritional deficiency is unknown in man, and such deficiencies as may occur are indirect and related to disease states, intoxications, and disorders of metabolism as have been mentioned under biotin.

Trace Elements. These include some thirty-five or forty minerals which are found for the most part in minute amounts in foods and in the tissues of animals (including man). Some, such as iron and iodine, which might otherwise be included in this group, have already been discussed in separate chapters because a deficiency of them produces well-recognized clinical syndromes. Of the remainder it should be noted that such remarks as are made about possible essential rôles in human nutrition and the effects of their deficiency must be considered as entirely tentative.

Fluorine has been of greatest interest clinically as a toxic substance, which, when present in too great concentration in the drinking water produces mottled dental enamel or fluorosis Even as little as one part per million in drinking water will produce the disease when such water is taken continuously during the time that the permanent teeth are primary On the other hand recent studies suggest that a small amount of fluorine is necessary for the proper formation of dental enamel

Fluorine as well as most of these trace elements is extremely poisonous in high concentration but as pointed out in the introduction these amounts are many many times greater than those presumably needed by the body and hence the general rule that essential food substances are harmless in ordinary (biologic) amounts is maintained

Copper has already been discussed briefly in the chapter on Iron Deficiency Copper is concerned in the extremely important, fundamental, intracellular oxidation mechanism and hence there is little doubt that it is essential to normal health and nutrition This view is supported by the high concentration of copper in the liver of the infant just before birth and the parallelism which exists between the metabolism of it and iron On the other hand it is very doubtful that a deficiency of copper exists in man except occasionally Certainly it does not occur without a coincident severe deficiency of iron Diets which supply an adequate amount of iron will usually supply enough copper and if iron is given therapeutically enough copper is ordinarily included to provide for the relief of a deficiency of the latter Such studies of the requirements of copper as have been reported indicate that an adequate daily intake ranges from 1 0 to 2 5 mg for infants and children to 3 5 mg for adults

Manganese deficiency in chickens is associated with perosis, a disease of the bone, and in rats is said to cause sterility of the males These experimental findings as well as the

occurrence of manganese in considerable amounts in some human organs has suggested that it is a necessary component of the human diet No clinical expression of manganese deficiency is known, however Somewhat similarly **cobalt** appears to be an important and essential element in the nutrition of sheep and cattle and a deficiency of it is apparently the cause of coast disease" or "Morton Maine s disease which is prevalent in Australia and New Zealand Again, no human counterpart is known to exist

A lowering of the **magnesium** content of the body, as reflected by the concentration in the blood, to a sufficient degree produces symptoms and if great enough, results in increased irritability, tachycardia, convulsions, and death There is no evidence, however, that a dietary deficiency of magnesium occurs in man The related elements, **barium, strontium,** and **beryllium** have no known place in animal nutrition, and beryllium has not been reported as a normal constituent of tissue Experimentally, however, it can be used to produce a severe experimental rickets The use of barium in x ray examinations and its relative lack of toxicity when taken by mouth are well known Certain variations in the amount of **zinc, tin,** and **arsenic** in different organs as well as in certain secretions suggest some specific function of these substances in animal nutrition What this may be is unknown and no characteristic manifestation of a deficiency of these substances is known in either experimental animals or man

There is little experimental and no clinical evidence that **aluminum,** or **vanadium** have any essential part in nutrition **Selenium** resembles fluorine in that it is best known for its toxic action In sufficient concentration in feed it causes "alkali disease in cattle No effect of a deficiency of it is known **Bromine** which is more closely related to fluorine shows certain local concentrations in the body which suggest

specific nutritive functions but no deficiency disease, corre sponding to that of iodine in relation to the thyroid, is known Much the same may be said of **nickel, silver,** and **mercury,** all of which are found in the human body in small amounts **Lithium, rubidium** and **cesium** are related to the alkali metals, sodium and potassium, which are of exceeding importance in nutrition and which are required by the body in large amounts The former, however, have no such impor tant role and there is little evidence so far to indicate the part they play, if any, in human physiology and hence nutrition

Silicon, one of the most abundant elements, is found in various organs and tissues of the human body but has no known significance in nutrition, nor has **boron,** another abundant and widely distributed element Many other ele ments, such as **lead, titanium, cerium, thorium,** and **zir conium,** are found at times in animals and human tissues but little is known of the constancy or significance of their presence In any event it is likely that the requirement for them, as well as for many of the other elements listed above are so small that a deficiency of any of them occurs only experimentally

There are certain substances which are needed in rather large amounts but are rarely if ever deficient because they are supplied adequately by the diet These include sodium, potassium, phosphorus (phosphates), and chlorides Phos phorus has already been discussed in the chapter on Vita min D and Calcium Deficiency Deficiencies do occur but only rarely are they strictly of dietary origin Instead they are the result of abnormal and greatly increased losses which makes the intake insufficient This is seen acutely in cases of vomiting and diarrhea with a loss of chlorides in the secre tions It also occurs with excessive sweating when water alone (unsalted) is used to replace the fluid Both of these

circumstances produce well known syndromes A somewhat similar condition may perhaps exist in a more chronic form These states can, however, scarcely be called deficiencies in the usual sense of the word and it must be extremely un common that nutritional deficiencies of sodium, potassium, phosphorus, and chloride exist

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A Summary of the Vitamins and the Laboratory Diagnosis of Deficiency Diseases

Section I A Tabular Summary of the Vitamins

Section II The Principal Dietary Sources of the Essential Food Factors

Section III Laboratory Methods for Diagnosing Deficiency Diseases

This section is designed to accomplish a three fold purpose First there is presented a tabular summary of the various vitamins The summary of the information relative to each vitamin is arranged in accordance with the plan followed in each individual chapter of the text

Second there is a tabular presentation of the principal dietary sources of the various essential food factors No detailed tables of the vitamin content of foods are included But for those who desire this quantitative information references to suitable sources are given

Third, the laboratory methods now available for assaying the nutritional status of an individual are collected together and de scribed in detail The sequence of the methods for the diagnosis of the various deficiency states conforms to the arrangement of these deficiencies in the text

Section I

A TABULAR SUMMARY OF THE VITAMINS

VITAMIN A

Nature	A complex higher alcohol with unsaturated structural bonds Formed by animal metabolism from plant precursors the carotenes or provitamins Beta carotene is the most important provitamin Vitamin A is fat soluble relatively heat stable, relatively well stored by the body
Function	Participates in maintaining the health of epithelium Participates in the formation of visual purple Essential to proper growth
Dietary Source[1]	*Excellent* Fish oils liver egg yolk cheese, butter, spinach turnip greens lettuce mustard greens green beans carrots *Fair* Whole milk, asparagus, okra yellow corn meal
Pathology	Keratinization of epithelium and replacement of specialized epithelium by undifferentiated stratified and cornified surfaces Altered visual purple formation
Clinical Expression	Night blindness Xerosis and Keratomalacia Dermatosis Vaginitis Often complicated by bronchiectasis pneumonia etc mostly in children
Diagnosis	Diet history Early clinical signs and symptoms Adaptometer tests (tests of night blindness) Blood serum vitamin A and carotene level Therapeutic trial
Treatment[2]	Preventive 1500 to 5 000 I U daily Protective (children pregnant and lactating women etc) 6 000 to 8 000 I U daily Curative 10 000 to 40 000 I U daily
Common Units of Measure	1 U S P unit = 1 International Unit = The activity of 0 6 microgram of beta carotene = 1 4 Sherman Munsell Units

[1] The provitamin A content (beta carotene etc) of the foods listed has also been considered in evaluating the importance of the foods as a dietary source of vitamin A

[2] Preventive protective and curative amounts are used in the same sense as in the text i e preventive generally reflects requirements protective refers to amounts suggested for pregnant and lactating women growing children and others whose requirements are increased and curative doses are those commonly employed therapeutically in ordinary clinical expressions of the deficiency It should be emphasized that these amounts are only suggestions The therapy for a specific individual should be conditioned by the various factors that affect absorption utilization and the need of the individual These factors are adequately discussed in the text

THIAMIN (VITAMIN B_1)

Nature	A pyrimidine thiazole compound Only known vitamin containing sulphur Water soluble Heat labile Poorly stored in the body
Function	Necessary for the proper utilization and metabolism of carbohydrates (essential to a co enzyme system concerned with the intermediary metabolism of carbohydrates) Essential for the maintenance of normal nerves psyche appetite and digestion circulation and normal growth
Dietary Source	*Excellent* Lean pork peanuts dried peas and beans soy beans oatmeal *Good* Liver chicken (dark meat) beef green beans and green peas walnuts pecans chestnuts whole grain wheat or rice
Pathology	Degeneration of the peripheral nerves muscle atrophy cardiac dilatation and failure, serous effusions
Clinical Expression	Beriberi Neuralgia and neuritis weakness paralysis muscle atrophy Cardiac enlargement and failure tachycardia edema serous effusions Anorexia indigestion constipation
Diagnosis	Diet history Calculation of the vitamin B_1 adequacy of a diet by the use of the formula and prediction chart of Cowgill or by the formula of Williams and Spies Clinical symptoms and signs Determination of the urinary excretion by biologic or chemical methods and blood pyruvic acid Therapeutic trial
Treatment	Preventive Approximately 0 5 to 3 mg daily Protective Approximately 3 to 5 mg daily Curative Approximately 10 mg daily
Common Units of Measure	1 mg thiamin (B_1) = approximately 333 International Units = approximately 600 Chase Sherman Units

NICOTINIC ACID

Nature	An organic compound chemically related to nicotine but having very different pharmacologic properties Heat stable Water soluble
Function	A constituent of a coenzyme system necessary for normal cellular life Exact mechanism unknown but undoubtedly bears a relation to the health of epithelial and nervous tissue
Dietary Source[1]	*Excellent* Liver, lean pork, lean beef *Good* Milk egg yolk, spinach lettuce, green cabbage, turnip greens green peas, soy beans
Pathology	Dermatitis, inflammatory reactions of mucous membranes Possibly degenerative changes in the brain and spinal cord
Clinical Expression	Dermatitis stomatitis, gastroenteritis and diarrhea Proctitis and vaginitis Neurasthenia Psychosis Possibly combined sclerosis of spinal cord
Diagnosis	Diet history Early clinical symptoms and signs Possibly urinary excretion of nicotinic acid, and F_1 and F_2 substances Therapeutic trial
Treatment	Preventive Approximately 5 to 25 mg daily Protective Approximately 50 mg daily Curative Approximately 150 300 mg daily
Common Units of Measure	The milligram unit is the only common measure of dosage

[1] The exact content of nicotinic acid in various foods is still uncertain The foods listed are those shown to have a therapeutic or preventive effect on human pellagra and canine black tongue

RIBOFLAVIN (VITAMIN B_2 OR G)

Nature	A flavin compound having a yellowish green fluorescence Water soluble Heat stable Extremely liable to deterioration when exposed to light
Function	A constituent of a coenzyme system necessary for the proper utilization of carbohydrates Causative mechanism in the production of epithelial changes unknown
Dietary Source	*Excellent* Liver lean meats egg yolk peanuts *Good* Peas lima beans spinach lettuce carrots cauliflower, turnip greens pears peaches beets whole milk buttermilk whole grain wheat
Pathology	Inflammatory reaction of epithelium Vascularization of the cornea
Clinical Expression	Cheilosis and angular stomatitis Glossitis Keratitis Dermatitis
Diagnosis	Diet history Symptoms and signs Slit lamp examination of the cornea Therapeutic trial
Treatment	Preventive Approximately 0 5 to 3 mg daily Protective Approximately 3 to 5 mg daily Curative Approximately 3 15 mg daily
Common Units of Measure	1 mg = approximately 400 Sherman Bourquin Units

ASCORBIC ACID (VITAMIN C)

Nature	A lactone of a hexuronic acid Water soluble Heat labile Poorly stored in the body
Function	Essential for the formation and maintenance of normal intercellular ground substances particularly for the maintenance of intact blood vessels and for the formation of collagen
Dietary Source	*Excellent* All citrus fruits (oranges, lemons grapefruit, et cetera), turnip greens spinach turnips, cabbage tomatoes, green peas *Good* Cucumbers, sweet potatoes green beans onions
Pathology	Defective formation of proper intercellular ground substances Resultant imperfect bone formation increased capillary fragility cutaneous and mucous membrane hemorrhages
Clinical Expression	Scurvy Gingivitis subcutaneous and mucous membrane hemorrhages sore extremities lassitude slight edema anemia
Diagnosis	Diet history Symptoms and signs Vitamin C content of the blood plasma and leukocytes Urinary excretion of vitamin Saturation tests Capillary fragility tests Therapeutic trial
Treatment	Preventive Approximately 50 100 mg daily Protective Approximately 100 mg daily Curative Approximately 200 400 mg daily
Common Units of Measure	1 mg = 20 International Units = 2 Sherman Units

VITAMIN D AND CALCIUM

Nature	Activated sterols closely related to cholesterol Occur naturally in both the animal and vege table form Fat soluble Relatively heat stable Reasonably well stored in the body
Function	Facilitates proper absorption of calcium and phosphorus Important factor in normal bone formation
Dietary Source[1]	*Excellent* Fish liver oils egg yolk *Good* Salmon sardines butter cream
Pathology	In children imperfect bone formation resulting in rickets In adults rarefaction and softening of bones resulting in osteomalacia Low serum calcium which may initiate tetany in either children or adults
Clinical Expression	Rickets Irritability weakness deformities pot belly Osteomalacia Deep boring aching pain de formities Tetany Carpopedal spasm convulsions
Diagnosis	Diet history Symptoms and signs X ray Blood serum phosphorus phosphatase activity cal cium
Treatment	Preventive and Protective Approximately 400 1200 International Units daily Curative Approximately 1200 5000 Internation al Units daily
Common Units of Measure	*1 International Unit = 1 U S P Unit = approxi* mately 3 3 Steenbock Units

[1] Generally the amount of available effective sunlight is a more important source of Vitamin D for humans than is the dietary source

VITAMIN E

Nature	A complex alcohol, occurs naturally in more than one biologically active form α tocopherol being the most active of those studied Heat stable Fat soluble
Function	Uncertain Perhaps essential to nuclear function and to the health of rapidly multiplying and differentiating tissues Possibly interrelated with hypophyseal and ovarian functions
Dietary Source	Widely distributed in nature but exact content of various foods unsettled at present Particularly abundant in the oil of seeds such as wheat and cotton also in all leafy vegetables and to some extent in meats and eggs
Pathology	Exact pathological expression in humans uncertain Possibly related to failure of reproductive functions and to degenerative nerve disorders
Clinical Expression	Uncertain Possibly certain instances of habitual abortion *amyotrophic lateral sclerosis* and certain muscular dystrophies are expressions of vitamin E deficiency
Diagnosis	Therapeutic trial Dietary evaluation unsatisfactory at present and no well developed clinical tests
Treatment	Requirements and amounts of vitamin E necessary for therapeutic purposes still uncertain Doses of 4 15 cc of wheat germ oil daily or of 3 12 mg of α tocopherol daily have been frequently employed
Common Units of Measure	No reference standards 3 mg α tocopherol = approximately 4 cc of wheat germ oil (unconcentrated)

VITAMIN K

Nature	*A substituted derivative of naphthoquinone* Occurs naturally in more than one biologically active form Heat stable Fat soluble Apparently not well stored
Function	Necessary for the adequate production of pro*thrombin prothrombin is essential for normal* blood clotting
Dietary Source	Widely distributed but the exact vitamin K content of various foods unknown Occurs in kale spinach carrot tops tomatoes and liver Also formed by the action of intestinal bacteria and it is possible that the human is independent of the dietary supply
Pathology	Decreased prothrombin content of blood with resultant prolonged clotting time and hemorrhagic tendencies In adults most often conditioned by a deficiency of bile salts in the intestine and a resultant poor absorption of vitamin K
Clinical Expression	Hemorrhagic disease of the newborn Bleeding and hemorrhages associated with obstructive jaundice sprue celiac disease et cetera
Diagnosis	*Symptoms and signs Determination of the blood* prothrombin content Therapeutic trial
Treatment	The requirements for vitamin K have not been clearly determined 1 0 to 2 0 mg of the synthetic 2 methyl 1 4 naphthoquinone daily is *a satisfactory therapeutic dose When there is* a deficiency of bile salts in the intestine (as in obstructive jaundice) bile salts should also be given if the vitamin is taken orally
Common Units of Measure	1 mg 2 methyl 1 4 naphthoquinone = approximately 2 000 Ansbacher Units = approximately 1 700 Thayer Doisy Units = approximately 700 Almquist Units = approximately 25 000 Dam Units

Section II

THE PRINCIPAL DIETARY SOURCES OF THE ESSENTIAL FOOD FACTORS

The foods listed in these tables are classified as to their nutrient content on a basis of equivalent weights Obviously certain foods listed as merely good sources become excellent sources if a large amount of the food is eaten conversely a food classified as an excellent source of a nutrient actually is not an excellent source for the individual if eaten sparingly

Food Factor	Animal Products		Vegetables	Fruits	Cereals	Nuts
	Meats, etc	Dairy Products				
Vitamin A *						
Excellent source	Fish oils liver, egg yolk	Cheese, butter	Turnip greens, spinach, lettuce, mustard greens, carrots, green beans	Cantaloupe (deep colored), yellow peaches		
Good source		Milk, cream	Asparagus, okra, green peas	Bananas	Yellow corn meal	
Vitamin B_1 (Thiamin)						
Excellent source	Lean pork		Yeast dried beans and peas of all types Soy beans		Oatmeal whole grain	Peanuts
Good source	Lamb liver, chicken (dark meat) beef, egg yolk	Milk	Green peas and green lima beans		Enriched bread wheat, whole rice (brown) cornmeal	Walnuts, pecans, chestnuts
Nicotinic Acid**						
Excellent source	Liver lean pork lean beef					Peanuts
Good source	Egg yolk viscera	Milk	Yeast lettuce green cabbage turnip greens green peas soy beans		Enriched bread whole wheat whole rice (brown rice)	

* The provitamin A content (beta carotene etc) of the foods listed has also been considered in evaluating the importance of the foods as a dietary source of vitamin A

** The exact content of nicotinic acid in various foods is still uncertain The foods listed are those shown to have a therapeutic or preventive effect on human pellagra and canine black tongue

Riboflavin						Peanuts
Excellent source	Liver lean muscle meats egg yolk	Cheese				
Good source		Whole milk buttermilk	Yeast turnip greens mustard greens peas lima beans spinach lettuce carrots cabbage beets cauliflower	Peaches pears prunes	Whole grain wheat	
Vitamin C (Ascorbic Acid)						
Excellent source	Liver brains		Turnip greens mustard greens spinach turnips brussels sprouts cauliflower cabbage tomatoes green peas broccoli	Oranges lemons, grapefruit tangerines, strawberries, gooseberries raspberries cantaloupe		
Good source			Cucumbers potatoes (whole) green beans onions artichokes	Cherries bananas pineapples peaches apples, watermelon avocado		
Vitamin D[1]						
Excellent source	Fish liver oils egg yolk					
Good source	Salmon sardines	Butter, cream				

[1] Generally the amount of available effective sunlight is a more important source of vitamin D for humans than is the dietary source

Food Factor	Animal Products		Vegetables	Fruits	Cereals	Nuts
	Meats, etc	Dairy Products				
Vitamin E	Widely distributed in nature but exact content of various foods unsettled at the present Particularly abundant in the oil of seeds such as wheat and cotton, also in all leafy vegetables and to some extent in meats and eggs					
Vitamin K[1]	Widely distributed in nature but exact vitamin K content of foods unknown Occurs in kale, spinach, carrot tops, tomatoes, and liver Also formed by the action of intestinal bacteria, and it is possible that the normal human is independent of dietary supply					
Calcium						
Excellent source		Milk (either whole, dried, evaporated, condensed buttermilk), cheese				
Good source	Egg yolk		Green beans kidney beans, cabbage carrots, lettuce okra, turnips turnip greens mustard greens broccoli			

[1] It should be emphasized that sufficient bile salts must be present in the intestine if vitamin K is to be properly absorbed Actually an inadequacy of bile salts (obstructive jaundice etc) and consequent poor absorption is probably the most frequent clinical cause of this deficiency in adults

Protein						
Excellent source	All lean meats, including poultry, fish, et cetera Eggs	Milk Cheese				
Good source			Yeast all types of peas and all *types of* beans particularly soy beans			All types of nuts
Iron						
Excellent source	Liver, all lean meats, egg yolk		Kidney beans lima beans mustard greens spinach turnip greens	Apricots (dried) peaches (dried)	Wheat bran	
Good *source*	Brains		Green beans, broc coli brussel sprouts cabbage, lettuce green peas	Dates figs, prunes, raisins	Whole corn meal oat meal, whole wheat flour	
Iodine						

In general a dietary adequacy of iodine does *not* depend upon the kind of food ingested but upon the adequacy of iodine in the soil and water of the region where the food stuff is obtained There is one notable exception to this all sea foods contain ample iodine since they always have an adequate supply of iodine from sea water

For those who desire more detailed information as to the nutritive composition of foods, the following sources are suggested

Booher, Lela E , and Eva R Hartzler The Vitamin B_1 Content of Foods in Terms of Crystalline Thiamin U S Department of Agriculture, Technical Bulletin No 707, December, 1939

Booher L E , E R Hartzler, and E M Hewston A Compilation of the Vitamin Values of Foods in Relation to Processing and Other Variants U S Department of Agriculture, Circular No 638, May 1942

Chatfield C , and G Adams Proximate Composition of American Food Materials U S Department of Agriculture, Circular No 549 June 1940

Fixen, Margaret A Boas, and Margaret Honora Roscoe Tables of the Vitamin Content of Human and Animal Foods Nutrition Abstracts and Reviews 7 823 876, 1937

Munsell, Hazel E Vitamins and Their Occurrence in Foods The Milbank Memorial Fund Quarterly 28 311 344 October, 1940

Rose, Mary Swartz A Laboratory Handbook for Dietetics New York Macmillan, 1939

Taylor C M *Food Values in Shares and Weights* New York, Macmillan, 1942

Tepley, L J F M Strong and C A Elvehjem The Distribution of Niacin in Foods Jour Nutrit , 23 417 425 1942

Section III

THE DIAGNOSIS OF DEFICIENCY DISEASES BY LABORATORY METHODS

Until comparatively recently the diagnosis of the deficiency diseases has been made only by the evaluation of the dietary history, *the presenting symptoms and signs and the results of a therapeutic* trial This approach is still a necessary and clinically sound one but in recent years certain laboratory procedures have been developed which augment our diagnostic approach

Some of these laboratory procedures have been in use for many years and have been adequately studied and evaluated Others have been developed only recently and may be subject to modifications in accordance with our progress in the field of deficiency diseases Certain of the diagnostic tests described below require special laboratory facilities and are obviously not adaptable, in their present form to use in the average laboratory Yet it seems worthwhile to assemble here those tests which at the present time are most helpful in the diagnosis of deficiency diseases and to describe them in detail

VITAMIN A

Many observations have suggested that night blindness (nyctalopia) may be the result of vitamin A deficiency For this reason many instruments have been designed in an attempt to determine the presence of abnormal dark adaptation and to utilize this procedure diagnostically Many instruments have been devised but probably the most accurate of these is the Adaptometer of Hecht and Shlaer [1] The technique of the test as performed with this instrument will be described below

A diagnostic method more recently proposed as a means of evaluating an individual s status as regards vitamin A adequacy is the determination of the blood serum (or plasma) vitamin A and carotenoids There are many methods available for this, but the one to be described here is May s modification of the method of McCoord and Luce Clausen This method has the principal advantage of being applicable to a relatively small amount of blood and has proved reasonably satisfactory in studies conducted by the author

The Adaptometer Test The principal features of the instrument are a bleaching light of about 1500 millilamberts intensity a fixation light within the instrument which allows a constant area of the retina to be tested (this area is located 7° nasally from the eye and is 3° visual angle in diameter) a diaphragm which enables one to vary the time of exposure to the test light and a system of filters for varying the intensity of the test light *The same lamp serves as a bleaching light and a test light source* and the voltage to this lamp is carefully controlled by a voltage regulator The fixation lamp is a small dim light operating on a dry cell battery source of power

The right eye of the subject to be examined is exposed to the bleaching light (about 1500 millilamberts intensity) for 3 minutes (The intrinsic intensity of the bleaching light is determined by comparison with a standard light source and then modifying this intensity if necessary by introducing appropriate filters) During the bleaching period the subject fixes his attention on a small dot on the lens This enables one to bleach that area of the retina which will subsequently be localized by the fixation light during the period of regeneration of visual purple The optical system is then changed by a lever and the bleaching light now serves as a testing

light The intensity of the fixation lamp is adjusted so that the individual can see it as a very dim light As the test period progresses it may be necessary to lower this intensity as the subject s ability to see in the dark improves The blue filter is now placed in the path of light transmission This remains in its place throughout the test Another filter one selected to allow considerable light trans mission, is placed in the path of the test light The subject fixes his right eye on the dim fixation light and the test lamp is exposed in flashes of one fifth second duration with a frequency of about one flash every two seconds During the course of the test light flashes the operator gradually increases the intensity of the test light by *altering a variable wedge fixed in the course of the transmitted light* The subject reports the *first* appearance of a visible flash and this value is recorded in terms of the filter used, the setting of the wedge and the time of the determination This procedure is repeated ap proximately 15 times during a thirty minute period after the bleach ing period is complete Obviously during the course of the thirty minutes one s ability to see dim light improves and the operator must change his filters to decrease progressively the amount of light transmitted The operator must also take care that the fixation light does not become too bright due to this progressive regenera tion of visual purple The subject must be thoroughly aware of the necessity of focusing his eye on the fixation light during the test flashes and of reporting the very *first* flash of light discernible

The absolute values of the intensity of the light perceived can be determined by correcting the known intensity of the test light for the filter used and the wedge setting For convenience this value is expressed in terms of the logarithm of the intensity of the light perceived The necessary tables for making this conversion are sup plied with each individual instrument

Hecht and Mandelbaum feel that a normal individual should perceive an intensity of light the logarithm of which is 3 5 micro microlamberts or less after the test [2]

The Determination of Vitamin A and Carotene in Blood Serum [3*] *Method* The method of preparation of extracts of material for analysis employed is that described by McCoord and Luce Clausen[4] except that the reagent is made with 22% of antimony trichloride in chloroform An Evelyn photoelectric colorimeter is used [5]

* Adapted from The American Journal of Diseases of Children through the courtesy of the authors and publisher

Reagents and Solutions

1 Antimony Trichloride Solution 22% solution of antimony trichloride in chloroform

2 Purified Petroleum Benzene U S P (Petroleum ether)

3 95% Ethyl Alcohol

Procedure To 1 cc of serum (or plasma) in a narrow mouth tube * 2 cc of 95% ethyl alcohol is added and the mixture shaken Then 2 cc of purified petroleum benzene U S P (petroleum ether) accurately measured is added After the tube is tightly stoppered it must be shaken vigorously and continuously for ten minutes if complete extraction is to be attained Centrifuging causes the separation of the clear upper layer of purified petroleum benzene extract which is used in the analyses The layer measures 2 cc and from it 1 cc may easily be taken for the analysis

Analysis of the Extracts The yellow color of the petroleum benzene extract is due to carotenoid pigments the bile pigments being unextracted under these conditions The principal carotenoids in blood serum are carotene and xanthophyll other carotenoids are present in only meager amounts Furthermore the carotene and xanthophyll are usually present in the same proportion so that measurement of the total carotenoids gives a sufficiently accurate reflection of the amount of carotene present for practical purposes Estimation of the total amount of carotenoids is necessary in the determination of vitamin A, in order that the measurement of the total blue color formed by the Carr Price reaction may be corrected for that portion arising from the carotenoids

Center Settings Filter 440** is placed in the microunit of the Evelyn photoelectric colorimeter The center setting of the instrument for 1 cc of purified petroleum benzene in a 1 cc open type microabsorption cell is determined in the manner described in the directions for operation of the instrument

A similar center setting is determined for the antimony trichloride reagent with Filter 620 It is important to redetermine the center settings immediately before each analysis One cc of the reagent should be accurately pipetted into a 1 cc open type cell The

* Extraction tubes Loss of the volatile extracting solvent during the extraction procedure would introduce serious error consequently precautions to prevent evaporation are essential Best quality cork stoppers should be used

** Filter numbers refer to their maximum transmission that is No 440 at 440 millimicrons and No 620 at 620 millimicrons

pipette used to measure the antimony trichloride reagent should be calibrated to deliver exactly 1 cc within four seconds by gravity with its tip steadied against the side of the cell 3 mm from the top, the last drop being blown out with a gentle puff This is to insure accuracy and rapidity in the technic and to avoid spilling of the liquid or immersion of the tip of the pipette in the reacting mixture All operations must be performed quickly, but accurately and smoothly

Carotenoids With Filter 440 in place and the instrument set at the appropriate center setting for purified petroleum benzene, 1 cc of the petroleum benzene extract of the serum is accurately measured into a 1 cc open type cell in place in the cell holder The cell is immediately wound into the colorimeter and the galvanometer reading (G440) recorded This reading is used in calculating the total amount of carotenoids present

Vitamin A The 1 cc cell with its contents is carefully removed from the colorimeter without spilling any of the extract The petroleum benzene is now evaporated to dryness The cell containing the petroleum ether extract is placed in a large test tube in the bottom of which is a plug of cotton This large tube is fitted with a rubber stopper through which a bent glass tube is inserted and this tube is connected to the water pump Gentle aspiration is started and the large test tube containing the cell is placed in a water bath maintained at 40°C This procedure excludes air from contact with the oily residue containing the vitamin A and evaporation is complete in 15 to 20 minutes The outside of the cell is then wiped dry and polished with lens paper and the cell replaced in the cell holder of the colorimeter Filter 620 is put into the colorimeter, and the galvanometer is set at the center setting for the antimony trichloride reagent When everything is ready 1 cc of the antimony trichloride reagent accurately measured is pipetted into the cell and it is immediately wound into the colorimeter rapidly, but smoothly to avoid spilling of the cell s contents

The blue color comes to its maximum formation in a few seconds The galvanometer reading (G620) is taken at the point of maximum absorption of light that is, maximum color formation

Expression of Results Pure vitamin A was not available for calibration of the photoelectric colorimeter to allow the results to be expressed as U S P units or milligrams of vitamin A Therefore the results are expressed in abstract units directly related to the galvanometer readings and readily convertible into U S P

units when a suitable conversion factor is found Actually, if desired, the factor 8 61 may be used to convert the vitamin A units here obtained into International Units This may be desirable for comparative purposes The factor 8 61 is obtained from the combined factors 0 41 for converting Evelyn colorimeter L values ($L^{1\%}_{1\,cm}$) into $E^{1\%}_{1\,cm}$ and the value 2100 for converting $E^{1\%}_{1\,cm}$ into International Units of vitamin A [6] It must be remembered however, that this is not a true expression of the content in International Units this can be achieved only when pure vitamin A is available

The results obtained by using the Evelyn colorimeter, are expressed in terms of units calculated as follows

$$L = 2 - \log G \text{ (Galvanometer reading)}$$

$$\text{Units per cc} = L \times \frac{2}{\text{number of cc of serum analyzed}}$$

$$\text{Units} \times 100 = \text{units per hundred cc}$$

Results for carotenoids are designated as 440 units and for vitamin A as 620 units indicating the maxima of the light band by which the color intensity is measured

Calculation of 620 Units of Vitamin A The value of the 620 units of vitamin A is calculated by subtracting the number of 620 units of blue color contributed by the carotenoids from the total number of 620 units of blue color formed by the antimony trichloride reagent

Each 440 units of carotenoids produces 0 11 of a 620 unit of blue color with antimony trichloride This factor was obtained by determining the ratio of $\frac{440 \text{ units}}{620 \text{ units}}$ for mixtures of carotene and xanthophyll in the proportions usually presented in blood serum [7]

Interpretation In a study of normal children May[8] found the range of blood A values by this method to be 5 5 to 27 3 units (if converted by the factor 8 61 as previously discussed approximately 50 to 230 I U) per 100 cc of serum In studies on normal adults by the author the blood serum vitamin A will usually be 8 0 units or better (approximately 70 I U) per 100 cc of serum

The levels of the carotenoids in normal children covered a very wide range in May's study namely 3 1 to 75 7 units per 100 cc of serum This has also been found to be the case in adults although normal adults usually tend to show the higher values for carotenoids with more consistency than do the children

THIAMIN (VITAMIN B_1)

Methods commonly used at present for detecting thiamin deficiency are first an analysis of the diet and the application of Cowgill s prediction chart or the formula of Williams and Spies Second, the determination of the daily urinary excretion of thiamin by either biological or chemical means, with or without a load test to determine the degree of tissue content in a manner similar to that used for vitamin C (ascorbic acid) estimations

A Prediction of Thiamin Adequacy from Dietary Analyses

The Prediction Chart of Cowgill[8] Recognizing the relationship between caloric intake body weight and the intake of dietary thiamin Cowgill proposed a formula which he felt would mathematically reflect this relationship for any given species Cowgill s formula is of the following form

$$\frac{\text{Vitamin Intake}}{\text{Total Calories}} = \text{K vitamin} \times \text{weight in kilograms}$$

in which the Vitamin Intake is expressed as milligram equivalents, and K vitamin is the constant value to be substituted according to the species under consideration By applying this formula to a large number of dietary histories associated with human beri beri and those not associated with beriberi he composed the prediction chart shown as Figure 16

To determine whether a given diet is beriberi producing or not, one calculates the Vitamin $\left(\frac{\text{Thiamin in milligram equivalents}}{\text{Total caloric intake}}\right)$ ratio and determines the body weight of the individual in kilo grams Then utilizing the prediction chart one can determine the nature of the given diet If one uses Cowgill s prediction chart it is probable that the best results will be obtained if Cowgill s table of the thiamin content of foods (expressed in milligram equivalents) is also used It should be emphasized that a value indicating an intake adequate to prevent deficiency disease does not necessarily represent an optimal thiamin intake

The Prediction Formula of Williams and Spies[9] Williams and Spies have proposed a satisfactory prediction formula on the same

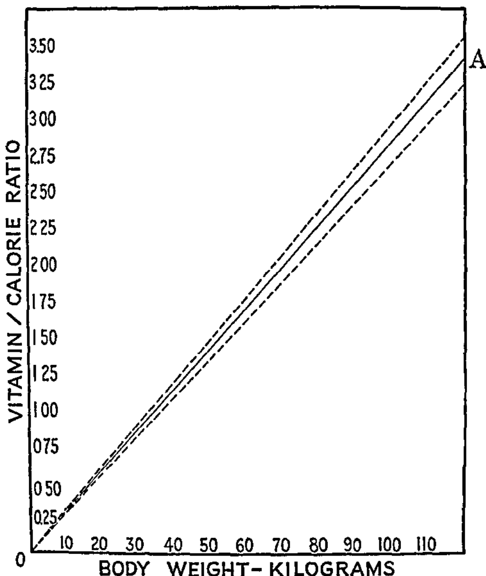


Fig 16 Prediction Chart for the Human Requirement of Vitamin B_1

The adequacy in vitamin B_1 content of a given human diet for individuals of different body weights may be estimated by reference to this chart The plot indicated by line OA represents the probable minimum vitamin B_1 requirement referred to body weight The area between the dotted lines represents a zone of uncertainty If the Vitamin/Calorie value of the diet for a given body weight falls definitely above line OA the ration is deemed adequate with respect to vitamin B_1 if the point proves to be appreciably below the line the vitamin requirement is not satisfied by this diet and beriberi should occur provided the period of subsistence on this ration is sufficiently extended if the plot is close to line OA or between the dotted lines the diet may be considered as "border line in character (*Reproduced through the courtesy of Dr G R Cowgill and the Yale University Press*)

fundamental principle employed by Cowgill The principal alteration made by Williams and Spies is the correction of the caloric intake to allow for the thiamin sparing action of fats In addition they have used tables of the thiamin content of foods that assign no value to fats *These tables are based on the cure* or prevention of polyneuritis rather than on anorexia prevention values, and larger allowances have been made for losses in cooking Then too, they do not feel that the weight of the individual is of enough importance in calculating adequacy of dietary thiamin to include in their formula The Williams and Spies formula is

$$K = \frac{\text{Thiamin Intake in micrograms}}{\text{Total Non-fat Caloric Intake}}$$

Using this formula a K value of 0 3 is considered a critical ratio *Diets yielding values below this figure are felt to be beriberi pro*ducing and those above are considered adequate to prevent the occurrence of beriberi although values slightly above a 0 3 ratio are clearly not optimal

B Methods for Determining the Urinary Excretion of Thiamin

Methods available for estimating the daily urinary excretion of thiamin are of two general types biological and chemical The three methods described below are the Fermentation Method of Schultz Atkin, and Frey (a biological method), and the chemical methods of Melnick and Field and of Hennessey and Cerecedo The chemical methods are more specific (they measure only thiamin) than the fermentation method On the other hand the fermentation method is more simple technically and is particularly useful if large numbers of determinations must be made

The Determination of Thiamin in Urine by the Yeast Fermentation Method [10]*

Reagents and Solutions

1 Solution A (buffer plus nicotinic acid)

Ammonium acid phosphate	180 Gm
Diammonium phosphate	72 Gm
Nicotinic acid	0 2 Gm
Distilled water to make	1 0 liter

* The details of the method described above are presented through the courtesy of Dr C N Frey of Standard Brands Incorporated

Solution A should be sterilized by heating at the temperature of flowing steam (Arnold sterilizer) for 30 minutes If desired the sterile buffer solution may be transferred to a sterile automatic burette and thereafter kept at room temperature If such a burette is not available the solution may be handled in the same manner as Solution B

2 Solution B (sugar and salts)

Dextrose (anhydrous)	200 0	Gm
Magnesium sulphate ($MgSO_4$, $7H_2O$)	7 0	Gm
Potassium acid phosphate	2 2	Gm
Potassium chloride	1 7	Gm
Calcium chloride ($CaCl_2$, $2H_2O$)	0 5	Gm
Ferric chloride ($FeCl_3$, 6H O)	0 01	Gm
Manganese sulphate ($MnSO_4$ $4H_2O$)	0 01	Gm
Distilled water to make	1 0	liter

Solution B should be distributed into 500 cc Erlenmeyer flasks (cotton plugged) and sterilized on three consecutive days for 30 minutes in an Arnold steam sterilizer or its equivalent Thereafter the flasks should be capped with foil or paper and then may be stored at room temperature When a flask has been opened (for use) it is thereafter kept in a refrigerator (4°C) until emptied

3 Thiamin Standard Solution Dissolve a weighed amount of thiamin chloride in distilled water to make a solution containing 0 1 mg of thiamin chloride per cc Make a subdilution to contain 0 001 mg or 1 gamma per cc This solution is distributed in 200 cc Erlenmeyer flasks (cotton plugged) Sterilize in the Arnold steam sterilizer for 20 minutes on two successive days and then treat as directed for solution B

4 Concentrated Sulfuric Acid

5 0 1 N Sulfuric Acid

6 0 1 N Sodium Hydroxide

7 1% Solution of Alazarin Red in Distilled Water

8 The Yeast Suspension Weigh 10 0 Gm of baker s yeast into a deep weighing scoop or a small beaker *Note* Do not use either the small foil wrapped cakes of yeast or the high vitamin B_1 baker s yeast Add distilled water to the yeast slowly at first until a smooth heavy cream has been produced and then enough to make the volume of the suspension 250 cc This suspension is made just prior to each assay run

The Apparatus The fermentometer consists of a water thermo

stat a shaking device, and a series of gasometers The reaction mixtures (yeast, sugar, et cetera) are placed in 250 cc wide mouth bottles fitted with one hole rubber stoppers These bottles are shaken at about 100 oscillations per minute The gas evolved is collected in the gasometers and is measured at atmospheric pressure and room temperature *

The degree of agitation of the reaction mixture (at 100 shakes per minute) depends upon the amplitude and other characteristics of the motion which is imparted to the bottles To be satisfactory the degree of agitation must not be so slow that the yeast settles out of suspension nor so violent that the solution is thrown against the stopper

The temperature of the water bath should be maintained at a temperature of 30 0 ± 0 2°C

The fermentometer as a whole is considered satisfactory for use in the present method when replicate tests (simultaneously made) show a variation in the three hour gas production of no more than 5 ml of gas

Procedure The 24 hour excretion of urine is collected in a bottle containing sufficient sulfuric acid to make the final volume of the urine acid to congo red paper Usually 20 drops of concentrated sulfuric acid is sufficient The total volume is measured and a satisfactory aliquot is chosen (approximately 100 200 cc) This aliquot is placed in a stoppered bottle and subjected to flowing steam (Arnold Sterilizer) for 30 minutes Care must be taken to make certain the sample is acid to congo red paper (pH 3 5 or lower) before the sample is sterilized After sterilization the sample may be stored on ice for at least 6 8 weeks without significant loss in its yeast fermentation stimulating properties

From this sterilized aliquot of the 24 hour specimen, a sample is chosen which theoretically will produce a stimulation to gas pro duction equivalent to 3 gamma of thiamin The size of this sample will be determined by the expected 24 hour urinary output in gamma and by the total volume of the 24 hour specimen This sam ple is then neutralized with 0 1 N NaOH and then made just acid by an additional drop or so of 0 1 N sulfuric acid Usually an in side indicator may be used for neutralization (impossible only when urine is too deeply pigmented) For this purpose 3 drops of a 1% solution of alazarin red are added to the urine and the titration car ried to the appearance of a pink color

* Fermentometers are now manufactured and may be purchased as a unit

The sample is now ready for the addition of the salt buffer and yeast solution as is described below

The Assay of a Single Unknown To each of three reaction bottles add 5 ml of Solution A and 15 ml of Solution B Then add the following

No 1 2 ml = 2 gamma thiamin standard solution plus 52 cc H_2O
No 2 4 ml = 4 gamma thiamin standard solution plus 50 cc H_2O
No 3 X ml original sample plus (54 – X) cc H_2O

To each bottle add 25 cc of the yeast suspension and place the bottles in the fermentometer Allow the bottles to be shaken at least 3 minutes while their contents are adjusting their temperature to that of the water bath Then make the initial reading on each fermentometer Note the time of this reading Determine the total volume of gas evolved in three hours for each

Estimate the fermentation activity in Test 3 by comparison with Tests 1 and 2 assuming a linearity of response between 2 gamma and 4 gamma of thiamin The fermentation activity is expressed in gamma of thiamin chloride For example

No	cc gas evolved in three hours	Fermentation Activity
1	350	2 gamma (known)
2	410	4 " (known)
3	383	3 1 " (estimated)

The sample (No 3) shows a fermentation activity of 3 1 gamma per x cc The thiamin content* of the total sample may be estimated from this value

The Hennessy and Cerecedo Modification of the Thiochrome Method for Determination of the Thiamin Content of Urine [11] †

Reagents and Solutions

1 Glacial Acetic Acid
2 25% Potassium Chloride Solution
3 1% Potassium Ferricyanide Solution
4 15% Sodium Hydroxide Solution
5 Isobutanol C P
6 Decalso approximately 50 mesh The Permutit Company New York City

* Actually one determines the amount of thiamin plus certain other yeast stimulating substances In spite of this lack of specificity the clinical interpretation of the results obtained seems quite valid

† Adapted from the Journal of the American Chemical Society through the courtesy of the authors and the publisher

Procedure Five to 20 cc of a 24 hour collection of urine containing up to 10 gamma of thiamin (as indicated by a rough direct assay) is adjusted to pH 4 0 to 4 5 with acetic acid It is brought to a boil and introduced into the exchange tube[16] whose temperature is kept near 100°C by passing flowing steam through an outer jacket surrounding the exchange tube The solution is allowed to pass through the decalso bed in the exchange tube in 3 to 5 minutes, and is followed by three 5 cc portions of boiling water The stopcock of the exchange tube is shifted to suction and the excess water is withdrawn from the tube

A boiling solution of 25% potassium chloride is now introduced into the funnel at the top of the exchange tube to remove the thiamin from the decalso bed The flow of the potassium chloride solution through the decalso bed should be regulated at about 1 0 cc per minute The amount of potassium chloride to be passed over the decalso bed and collected is governed by the expected amount of thiamin present in the original sample For 0 1 gamma collect 10 cc, for 1 3 gamma 15 cc, for 3 7 gamma 20 cc, and for 7 10 gamma 25 cc

Two 5 cc aliquots of the potassium chloride eluate are measured into two 25 cc glass stoppered graduated cylinders To the test sample 0 05 cc of 1% potassium ferricyanide is added Three cc of 15% sodium hydroxide solution is added with mixing and is immediately followed by 13 cc of isobutanol The mixture is shaken vigorously for one minute and centrifuged for one half minute to separate the two layers It is then carefully poured into a separatory funnel and the lower layer removed and set aside for comparison with the same solution in the blank determination The isobutanol layer is transferred to a test tube and 2 to 4 grams of anhydrous sodium sulfate is added After mixing well the sodium sulfate is allowed to settle and 10 cc of the perfectly clear isobutanol solution is decanted into the curette of the fluorometer The same procedure is repeated for the blank except that potassium ferricyanide is omitted and the order of the addition of the sodium hydroxide and the isobutanol is reversed

The degree of fluorescence of the unknown urine sample is now determined in the fluorometer This in turn is corrected for the fluorescence contributed by the blank sample (to which no ferricyanide was added) and similarly for the fluorescence contributed by an aqueous blank treated in the same manner but with no thiamin present

Known amounts of thiamin between the 0 10 gamma range are treated with alkali and ferricyanide and extracted as is described above After correction of these values the corrected fluorescence of the unknown sample may be compared with the known thiamin standards and the content of thiamin in the unknown urine calculated

It may be found convenient to prepare standard solutions of quinine sulphate *in 0 1* N *sulphuric acid and equilibrate their* fluorescence with known amounts of thiamin Subsequently one may use these same stable quinine sulphate standards for determining the thiamin content of unknown samples

Interpretation Studies[12 13] of the daily urinary excretion of thiamin as measured by the fermentation method indicate that normal men excrete *400* gamma or more of yeast stimulating substances daily whereas normal women excrete 300 gamma Children have much the same excretion By the chemical method[14 15 16] men excrete 90 gamma or more contrasted to the 60 or more gamma excreted by women by the same method

Load Tests In order to determine the tissue stores 1 mg of thiamin can be injected intramuscularly or intravenously and the excretion over a subsequent period determined By the fermentation method with 1 mg injected intramuscularly a normal individual will excrete 180 gamma or more of yeast fermentation stimulating substances during a succeeding 4 hour period[13] Determined chemically after 1 mg injected intravenously 120 gamma or more of thiamin will be excreted during the subsequent 4 hour period 100 gamma or more during the first half hour period[16]

Determination of Blood Pyruvic Acid[17]

Reagents and Solutions

1 10% trichloracetic acid

2 *0 1%* 2 4 dinitrophenyl hydrazine dissolved *in 2* N *hydro*chloric acid

3 Ethyl acetate pure

4 10% sodium carbonate

5 2 N sodium hydroxide solution

Procedure Blood is drawn from a vein with a minimum of stasis and without movement of the arm in a carefully cleaned dry *cold* 2 or 5 cc syringe The desired volume is carefully adjusted to the mark and the blood expelled quickly in a fine stream through the needle into *cold* 10% trichloracetic acid to make 5 volumes in a

cork stoppered flask or centrifuge tube The contents are mixed shaken, and centrifuged If necessary they may be kept *on ice* before or after centrifuging Three cc of the clear, supernatant fluid are added to 1 cc of the 2 4 dinitrophenyl hydrazine reagent in a centri fuge tube mixed and allowed to stand at room temperature for not less than 10 minutes The mixture is then extracted with 4 cc. of ethyl acetate, shaking in the corked tube After shaking, when the layers have separated the bottom trichloracetic acid layer is pipetted off with a capillary pipette and transferred to another cor responding tube (tube 2) *This trichloracetic acid portion is then* extracted twice with 2 cc portions of ethyl acetate, adding the ethyl acetate to the first portion in tube 1, the trichloracetic solution being *finally discarded after making sure no ethyl acetate remains on it* The ethyl acetate is extracted with exactly 2 cc. of the sodium car bonate solution for at least 3 minutes, being careful to avoid loss by effervescence during warm weather The lower sodium carbonate layer is pipetted off with a capillary pipette and placed in a clean corresponding tube tube 3 The extraction is repeated twice more and the combined sodium carbonate washings are extracted for the last time with 1 0 cc of ethyl acetate which is discarded The sodium carbonate solution is then transferred quantitatively to a clean tube and 4 cc. of 2 N sodium hydroxide are added In a warm climate this must be done in a cool room (20° C) and the sodium carbonate washings should be allowed to come into equilibrium with the sodium hydroxide kept in the room The mixture is al lowed to stand for 10 minutes until the color is stabilized Color is then determined in a photo-electric colorimeter with Wratten No 62 light filter (Evelyn 540) and the amount of pyruvic acid read off from a standard reference curve

NICOTINIC ACID

At the present time there are no very satisfactory methods available for determining by laboratory methods the status of an individual as regards nicotinic acid adequacy The diagnostic significance of *the methods* now in use for estimating nicotinic acid in blood and urine is not clear That of Perlzweig, Sarett and Margolis which is referred to in the text may be tried but its value is not yet fully established.

RIBOFLAVIN

Several methods are available for estimating the amount of riboflavin in blood and urine of humans but the significance of the results obtained has not been clearly established

An alternative approach for detecting riboflavin deficiency is the biomicroscopic examination of the eyes under slit lamp illumination Recent studies have indicated that vascular proliferation from the limbic plexus and the invasion of the anterior portion of the cornea by these proliferating vessels occurs in ariboflavinosis The visualization of the ocular evidences of ariboflavinosis is achieved by use of the ordinary slit lamp It is wise to use both the high power objective and ocular of the instrument for this purpose As much of the perimetry of the cornea as possible should be examined particularly since normally a few vessels may cross the corneoscleral junction and invade the anterior portion of the cornea in the inferior nasal quadrant In ariboflavinosis there is congestion of the limbic plexus with circumcorneal congestion vessels proliferate and invade the anterior portion of the cornea throughout its perimetry closely interlacing and anastomosing superficial nebulae may occur at the perimetry of the cornea More advanced and more chronic cases may show invasion of the posterior portion of the cornea and interstitial or posterior opacities may occur in these cases *A more adequate description of the ocular changes* occurring in ariboflavinosis may be obtained from the studies of Sydenstricker and his co workers[18] Caution should be used in interpreting minor grades of vascularization as evidence of general riboflavin deficiency and observations should be controlled by repeated examinations during treatment

ASCORBIC ACID (VITAMIN C)

Methods for estimating the nutritional status of an individual as regards ascorbic acid are of three general types (1) The determination of the ascorbic acid content of the blood with or without test dose (2) the determination of the urinary excretion with or without a test dose (3) tests designed to estimate the capillary fragility Obviously an increased capillary fragility is not always due to ascorbic acid deficiency yet if due regard for other conditions is maintained this test may be of clinical aid

The Determination of Ascorbic Acid in Blood[19]

Reagents and Solutions 1 Standard 2 6 dichlorophenolindo-

cork stoppered flask or centrifuge tube The contents are mixed shaken, and centrifuged If necessary they may be kept *on ice* before or after centrifuging Three cc of the clear, supernatant fluid are added to 1 cc. of the 2 4 dinitrophenyl hydrazine reagent in a centri fuge tube, mixed and allowed to stand at room temperature for not less than 10 minutes The mixture is then extracted with 4 cc of ethyl acetate shaking in the corked tube After shaking, when the layers have separated the bottom trichloracetic acid layer is pipetted off with a capillary pipette and transferred to another cor responding tube (tube 2) This trichloracetic acid portion is then extracted twice with 2 cc portions of ethyl acetate adding the ethyl acetate to the first portion in tube 1, the trichloracetic solution being finally discarded after making sure no ethyl acetate remains on it The ethyl acetate is extracted with exactly 2 cc of the sodium car bonate solution for at least 3 minutes, being careful to avoid loss by effervescence during warm weather The lower, sodium carbonate layer is pipetted off with a capillary pipette and placed in a clean corresponding tube tube 3 The extraction is repeated twice more and the combined sodium carbonate washings are extracted for the last time with 1 0 cc of ethyl acetate which is discarded The sodium carbonate solution is then transferred quantitatively to a clean tube, and 4 cc. of 2 N sodium hydroxide are added In a warm climate this must be done in a cool room (20° C) and the sodium carbonate washings should be allowed to come into equilibrium with the sodium hydroxide kept in the room The mixture is al lowed to stand for 10 minutes until the color is stabilized Color is then determined in a photo electric colorimeter with Wratten No 62 light filter (Evelyn 540) and the amount of pyruvic acid read off from a standard reference curve

NICOTINIC ACID

At the present time there are no very satisfactory methods available for determining by laboratory methods the status of an individual as regards nicotinic acid adequacy The diagnostic significance of the methods now in use for estimating nicotinic acid in blood and urine is not clear That of Perlzweig Sarett and Margolis which is referred to in the text may be tried, but its value is not yet fully established

RIBOFLAVIN

Several methods are available for estimating the amount of riboflavin in blood and urine of humans but the significance of the results obtained has not been clearly established

An alternative approach for detecting riboflavin deficiency is the biomicroscopic examination of the eyes under slit lamp illumination Recent studies have indicated that vascular proliferation from the limbic plexus and the invasion of the anterior portion of the cornea by these proliferating vessels occurs in ariboflavinosis The visualization of the ocular evidences of ariboflavinosis is achieved by use of the ordinary slit lamp It is wise to use both the high power objective and ocular of the instrument for this purpose As much of the perimetry of the cornea as possible should be examined particularly since normally a few vessels may cross the corneoscleral junction and invade the anterior portion of the cornea in the inferior nasal quadrant In ariboflavinosis there is congestion of the limbic plexus with circumcorneal congestion vessels proliferate and invade the anterior portion of the cornea throughout its perimetry closely interlacing and anastomosing superficial nebulae may occur at the perimetry of the cornea More advanced and more chronic cases may show invasion of the posterior portion of the cornea and interstitial or posterior opacities may occur in these cases A more adequate description of the ocular changes occurring in ariboflavinosis may be obtained from the studies of Sydenstricker and his co-workers [18] Caution should be used in interpreting minor grades of vascularization as evidence of general riboflavin deficiency and observations should be controlled by repeated examinations during treatment

ASCORBIC ACID (VITAMIN C)

Methods for estimating the nutritional status of an individual as regards ascorbic acid are of three general types (1) The determination of the ascorbic acid content of the blood with or without test dose (2) the determination of the urinary excretion with or without a test dose (3) tests designed to estimate the capillary fragility Obviously an increased capillary fragility is not always due to ascorbic acid deficiency yet if due regard for other conditions is maintained this test may be of clinical aid

The Determination of Ascorbic Acid in Blood [19]

Reagents and Solutions 1 Standard 2 6-dichlorophenolindo-

phenol Solution—Extract approximately 0 1 Gm of the dye (Eastman) twice with 25 cc portions of boiling water, pouring each extract through a small filter into a 50 cc volumetric flask (Reject insoluble residue) Cool and dilute to the mark This stock solution will keep about 2 3 weeks For daily use dilute 10 cc to 100 cc. with distilled water (recently boiled and cooled) Weigh accurately about 60 mg of ascorbic acid, dissolve and dilute to 100 cc with 5% acetic acid Dilute 2 cc of this solution to 50 cc with 5% acetic acid (approximating the concentration of ascorbic acid in deproteinized plasma) Now determine the ascorbic acid equivalence of the dye solution by titrating against 2 cc portions of the ascorbic acid solution The fresh ascorbic acid solution may be further checked by titrating against 0 01 N iodine solution (1 14 cc 0 01 N = 1 mg ascorbic acid) Standardized tablets of the dye are made by Hoffman La Roche, Nutley, New Jersey

2 5% *Metaphosphoric Acid Solution*

Procedure Pipette 2 cc of oxalated plasma into a 15 cc centrifuge tube, add 4 cc of distilled water and 4 cc of 5% metaphosphoric acid solution Mix thoroughly by tapping and centrifuge Transfer 2 cc portions of the supernatant fluid into round bottom centrifuge tubes and titrate with the standardized solution of 2,6 dichlorophenolindophenol using a 5 cc microburette (graduated in 0 01 cc divisions) The end point the first faint pink color which persists may best be observed in comparison with a blank solution

The concentration of ascorbic acid in the unknown sample of blood plasma may be determined in the following manner The amount of dye necessary to titrate the sample is expressed as the amount of ascorbic acid in milligrams (determined in the preparation of the solution) to which it is equivalent This value, if multiplied by $\frac{100}{0\ 4}$ equals the milligram content of ascorbic acid per 100 cc plasma

Interpretation The interpretation of the values obtained is adequately discussed in the text

Test Dose Blood samples are drawn before and 1 and 3 hours after the administration by mouth (preferably after a meal) of 600 mg of ascorbic acid (300 in the case of small children) either as crystalline ascorbic acid or an equivalent amount of orange juice whose ascorbic acid content has been determined by titration This may be combined with urinary load test (see below) Failure of the concentration in the blood to rise 2 mg per 100 cc or more, indicates a deficiency [20]

The Determination of Ascorbic Acid in Urine[19]

Reagents and Solutions 1 Standard 2 6 dichlorophenolindo phenol Solution The dye used for the urine titration should be twice the strength of that used for blood It is prepared just as described in the determination of blood ascorbic acid except that for daily use one dilutes 20 cc rather than 10 cc of the stock solution to 100 cc

Procedure The urine to be examined should be collected in dark tightly stoppered bottles and should be kept on ice previous to analysis * In addition each individual specimen should be acidified with glacial acetic acid (adding approximately 1 cc glacial acetic acid per 100 cc of urine) When possible the specimens should be analyzed within two hours of the time of voiding but if prepared as described above a 24 hour collection may be made without exces sive loss of reduced ascorbic acid

One cc of the acidified urine specimen is diluted with 10 cc of distilled water after which this is titrated with a standardized 2 6 dichlorophenolindophenol using a microburette (graduated in 0 01 cc divisions) The end point is the first faint pink color that persists

When the urine is highly colored it is often necessary to dilute the urine with more than 10 cc of water to obtain a satisfactory end point If a large amount of vitamin C is present in the specimen it may be necessary to use only 1 cc of the diluted specimen for titra tion by the dye In the latter case the factor of dilution must be considered in calculating the amount of ascorbic acid present in the urine

The amount of the dye necessary to titrate the sample is expressed as the amount of ascorbic acid in milligrams (determined in the preparation of the dye solution) to which it is equivalent This value indicates the amount of ascorbic acid in the sample After this is done the total 24 hour excretion of ascorbic acid is then able to be determined

Interpretation The significance of the values for excretion of ascorbic acid in the urine is discussed in the text

A Test of the Ascorbic Acid Content of Tissues (Saturation Test)

Test Dose A satisfactory method of testing tissue stores (satura tion) consists of administering orally preferably after a meal 600 mg of ascorbic acid (300 mg in the case of small children) either as

* All collection bottles and glassware used in determining ascorbic acid in blood or urine should be carefully cleaned with distilled water free from any trace of copper

crystalline ascorbic acid or an equivalent amount of orange juice whose ascorbic acid content has been determined by titration This may be combined with the blood level load test (see above) Following the administration of the test dose a total 24 hour specimen of urine is collected and analyzed as is described above Five cc of oxalated blood is obtained in the fasting state, and at one, three and six hours after giving the oral test dose The ascorbic acid content of these specimens is determined as described above

During the 24 hour period of urine collection, the diet should be reasonably low in ascorbic acid content

If the tissues contain a normal amount of vitamin C, approximately 20 30% of the test dose should be recovered in the 24 hour urine [21]

The Determination of Capillary Resistance The two most common methods of estimating capillary fragility are those of Gothlin[22] and Dalldorf [23]

Gothlin s Method A circular area of 60 mm in diameter is delineated on the skin over the anticubital fossa The arm is placed in the plane of the heart A sphygmomanometer cuff is placed about the arm at least 2 5 cm above the nearest part of the circular area and is inflated to a pressure of 50 mm Hg The pressure is *maintained for fifteen minutes The number of petechiae in the* circular area is then determined According to Gothlin a normal individual will have less than five petechiae present in this area More than eight petechiae is considered abnormal

Dalldorf s Method Negative pressure outside the capillaries may be used to induce petechiae as well as heightened pressures within as in the test of Gothlin Dalldorf employs a bicycle pump connected to a manometer which in turn is connected to a small glass cup whose inside diameter is 1 cm This glass cup is applied to the lateral aspect of the arm (not the forearm) Negative pressure is gradually increased over a period of one minute and the least negative pressure required to produce petechiae is determined This is checked by testing several areas in the same region In the region described negative pressures up to 30 mm Hg should not produce macroscopic petechiae

VITAMIN D AND CALCIUM

Laboratory evidence of vitamin D deficiency can be obtained by the determination of the blood serum calcium serum phosphorus, serum phosphatase activity and the x ray examination of the bones

Due to the intimate interrelation of vitamin D and calcium metabolism the same determinations are employed in the evaluation of a calcium deficiency As is the case in most laboratory procedures, a clinical evaluation of the individual is necessary to interpret the role played by either or both of the deficiencies in a given case

The *methods for determining the blood serum calcium* phosphorus and phosphatase activity are described below No attempt will be made to describe the x ray technic employed This is a familiar routine to the roentgenologist and is usually delegated to him

The clinical significance of the blood serum calcium phosphorus and phosphatase activity values and the x ray changes are adequately discussed in the chapter on vitamin D deficiency

Determination of Calcium in Serum by the Clark Collip Modification of the Kramer Tisdall Method [24] *

Reagents and Solutions 1 Potassium Permanganate Solution Dissolve 0 4 Gm pure potassium permanganate in 1 liter of redistilled water in a thoroughly clean Florence flask Insert funnel covered with a watch glass as a condenser and digest for several hours near the boiling point Cool let stand overnight and filter with gentle suction through a 3 inch Buchner funnel lined with ignited asbestos Transfer to a perfectly clean glass stoppered bottle and keep in a dark place This 0 01 N permanganate is standardized against 0 01 N sodium oxalate The permanganate solution should be frequently restandardized although after the first few days if *carefully prepared it should not deteriorate more than 0 1%* per week

2 0 01 N Sodium Oxalate Solution Dry highest purity sodium oxalate in an oven at 100-105° for 12 hours Dissolve exactly 0 67 Gm of the oxalate in redistilled water add 5 cc. of concentrated H_2SO_4 and dilute to one liter Mix well Transfer exactly 25 cc. of this solution to a 100 cc Erlenmeyer flask add 1 cc. concentrated H_2SO_4 and warm to about 70° and titrate with the $KMnO_4$ solution

3 4% Ammonium Oxalate Solution

4 Dilute Ammonia Solution Dilute 2 cc. of concentrated ammonia with 98 cc. of distilled water

5 1 0 N Sulfuric Acid

* The descriptions of the blood serum calcium phosphorus and phosphatase determinations have been adapted from Practical Physiological Chemistry by P B Hawk and O Bergeim P Blakiston and Son

Procedure Introduce into a graduated 15 cc centrifuge tube 2 cc of clear serum, 2 cc of distilled water, and 1 cc of 4% ammonium oxalate solution Mix thoroughly The centrifuge tube should have an outside diameter of 6 7 mm at the 0 1 cc mark Mixing is aided by holding the tube at the mouth and giving it a circular motion, tapping the lower end Let stand for 30 minutes or longer Again mix the contents Centrifuge for about 5 minutes at 1500 revolutions per minute Carefully pour off the supernatant liquid and while the tube is still inverted let it drain in a rack for 5 minutes resting the mouth of the tube on a pad of filter paper Wipe the mouth of the tube dry with a soft cloth Stir up the precipitate and wash the sides of the tube with 3 cc of dilute ammonia solution directed in a very fine stream from a wash bottle Centrifuge the suspension and drain again as before Add 2 cc of approximately normal sulfuric acid by blowing it from a pipette directly upon the precipitate so as to break up the mat and facilitate solution Place tube in a boiling water bath for about 1 minute Titrate with 0 01 normal potassium permanganate to a definite pink color which persists for at least one minute If necessary during the course of the titration warm the tube by placing in a water bath kept at 70 75° A micro burette graduated in 0 02 cc should be used

Calculation One cc of 0 01 N $KMnO_4$ *is equivalent to 0 2 mg* Ca

$$(X - b) \times 0\ 2 \times \frac{100}{2} = \text{mg Ca per 100 cc serum}$$

where X equals the number of cc of permanganate required in the titration, and b is the blank i e the number of cc of permanganate required to titrate 2 cc of the sulfuric acid solution to the usual end point

Determination of Serum Phosphatase and Inorganic Phosphate by the Method of Bodansky [25]

Reagents and Solutions 1 Substrate Into a 100 cc volumetric flask introduce successively 3 cc of petroleum ether (B P 20-40°C), about 80 cc of distilled water 0 5 Gm of sodium glycerophosphate 0 424 Gm of sodium diethyl barbiturate and water to volume (read at interface between petroleum ether and aqueous solution) Empty (out of doors) into a 100 cc glass stoppered pyrex bottle containing an inch layer of petroleum ether Keep in the refrigera tor When multiples of 100 cc are prepared it is advisable to distribute the substrate into small bottles

2 Stock Phosphate Solution 110 mg of potassium acid phosphate and 1 cc of concentrated sulfuric acid made up to 250 cc (1 cc. = 1 mg P)

3 Phosphate Standard Dilute 10 cc of stock phosphate solution to 300 cc Add a drop of toluene (6 cc = 0 02 mg P)

4 Acid Molybdate Solution (To be prepared fresh daily) Dilute while mixing cold 10 N sulfuric acid (kept in refrigerator) with an equal volume of 7 5% sodium molybdate solution The mixture should be free from even the slightest tinge of yellow To prepare the 7 5% sodium molybdate solution dissolve 90 Gm molybdic acid (ammonia and phosphate free) in 250 cc 5N NaOH in a 2 liter volumetric flask Dilute to volume and mix The solution should be faintly alkaline to phenolphthalein Let stand and decant for use

5 Dilute Stannous Chloride (To be prepared fresh daily) Dilute 1 cc of a 60% solution to 400 cc with water Keep in refrigerator between analyses The 60% solution is prepared by adding to 15 Gm stannous chloride sufficient concentrated hydrochloric acid to make a volume of 25 cc Keep in refrigerator and always prepare fresh monthly

Procedure Collect about 5 cc of whole blood in a centrifuge tube *allow to clot at room temperature remove clot then cen* trifuge (twice if necessary) The separated serum may be kept several hours in the refrigerator

Preparation of Filtrates (1) For serum inorganic P To 1 cc of serum in a test tube add 9 cc of 10% trichloracetic acid mix well and after a few minutes filter through 9 cm ashless filter paper (Whatman No 44) (2) For total inorganic P (serum inorganic P plus phosphate liberated by serum phosphatase from the substrate) Measure 10 cc of substrate preferably into a stoppered test tube Immerse *in a water bath at 37°C for a few minutes then add 1 cc* of serum with the tip of the pipette about 1 cm above the surface of the liquid tap to impart rotary motion so as to mix contents well and to wash down any serum on the side of the test tube Re place into water bath and remove after exactly one hour Cool in ice water for about 2 minutes add 9 cc of 10% trichloracetic acid mix let stand a few minutes and filter as above

When a high phosphatase activity is expected and a high dilution is therefore desired larger volumes of 10% trichloracetic acid may be employed

Preparation of Aliquots A wide range of concentrations of P in the aliquot is permissible, although the accuracy may be decreased from about 1 or 2% throughout most of the range to 3 to 5% at the limits In accordance with the concentration expected, aliquots of 2, 4 or 6 cc of filtrate are taken and made up with water to a total volume of 6 cc The comparison standards (2 or more in each

TABLE 1 (*Bodansky*) —INORGANIC P IN ALIQUOT AT STATED COLORIMETRIC READINGS CORRECTED FOR DEVIATION FROM BEER S LAW 0 02 MG STANDARD SET AT 20 MM
In Column D are given the values for decrements corresponding to an increase of the readings by 0 1 mm for use in interpolation

mm	0 *mg*	2 *mg*	4 *mg*	6 *mg*	8 *mg*	D
5	0 0974	0 0931	0 0892	0 0856	0 0823	18
6	792	764	737	711	687	12
7	665	645	626	607	588	9
8	570	554	539	525	512	7
9	499	487	475	464	453	6
10	443	433	424	415	406	5
11	398	390	382	374	367	4
12	360	353	346	340	334	3
13	328	322	316	311	306	3
14	301	296	291	286	282	2
15	278	274	270	266	262	2
16	258	254	251	248	244	2
17	241	238	235	232	229	1+
18	226	223	220	217	215	1+
19	212	209+	207	204+	202	1
20	200	198−	195+	193	191	1

mm	0 *mg*	2 *mg*	4 *mg*	6 *mg*	8 *mg*	D
21	0 01890	0 01869	0 01849	0 01829	0 01809	10
22	1790	1771	1753	1735	1717	9
23	1700	1683	1666	1650	1634	8
24	1618	1602	1587	1572	1557	8
25	1543	1529	1515	1501	1487	7
26	1474	1461	1448	1435	1422	7
27	1410	1398	1386	1374	1362	6
28	1351	1340	1329	1318	1307	6
29	1297	1287	1277	1267	1257	5
30	1247	1237	1228	1219	1210	5
31	1201	1192	1183	1174	1166	4
32	1158	1150	1142	1134	1126	4
33	1118	1110	1102	1094	1087	4
34	1080	1072	1065	1058	1051	4
35	1044	1037	1030	1023	1016	4
36	1010	1003	997	991	984	3

set) contain 0 02 mg of P (6 cc of the phosphate standard) Each set of determinations should include a number of check solutions to serve as additional standards These may be made by suitable dilutions of the stock phosphate solution varying the concentration from one half to three or four times that of the standard The re sults of the first determination usually fall within the permissible range If not repeat using an aliquot which will more nearly approach the P content of the standard

Colorimetric Procedure To each tube in sequence add 2 cc of acid molybdate solution* and mix by tapping in the same sequence

* The reagents should first be tested as follows To 6 cc of water in one test tube and an equal volume of 10% trichloracetic acid in another add 2 cc of acid molybdate solution and 2 cc of dilute stannous chloride solution mixing after each addition The blanks will be colorless or at most tinged faintly green or blue if the reagents are of suitable quality and properly prepared

add 2 cc of dilute stannous chloride solution to each tube and mix during the addition The color develops rapidly and comparison

TABLE 2 (*Bodansky*) —INORGANIC P CONTENT IN MG PER 100 CC CORRECTED FOR DEVIATION FROM BEER S LAW AND THE EFFECTS OF TRICHLOROACETIC ACID AND GLYCEROPHOSPHATE *

For 6 cc Aliquots (Or for 3 cc when half volumes are used)							For 4 cc Aliquots (Or for 2 cc when half volumes are used)						
mm	0 *mg*	2 *mg*	4 *mg*	6 *mg*	8 *mg*	D	mm.	0 *mg*	2 *mg*	4 *mg*	6 *mg*	8 *mg*	D
5	17 7	16 9	16 2	15 5	14 9	0 3+	*5*	25 8	24 7	23 7	22 7	21 8	0 5
6	14 4	13 8+	13 4	12 9	12 5	2+	*6*	20 9	20 1	19 4	18 8	18 2	3
7	12 0+	11 7	11 3	11 0	10 7	2−	*7*	17 6	17 1	16 6	16 1	15 6	2+
8	10 4	10 1	9 8	9 6−	9 3	1+	*8*	15 1	14 7	14 3	13 9	13 5	2
9	9 06	8 83	8 61	8 41	8 22	10	*9*	13 2	12 9	12 6	12 3	12 0	1+
10	8 04	7 87	7 70	7 54	7 38	08	*10*	11 7	11 5	11 2	11 0	10 7+	1+
11	7 23	7 09	6 95	6 81	6 67	07	*11*	10 5+	10 3	10 1	9 9	9 7	1
12	6 54	6 42	6 30	6 18	6 07	06	*12*	9 52	9 34	9 17	9 00	8 83	08+
13	5 96	5 86	5 76	5 66	5 56	05	*13*	8 67	8 51	8 36	8 22	8 09	07
14	5 47	5 38	5 29	5 21	5 13	04	*14*	7 96	7 83	7 71	7 59	7 47	06
15	5 05	4 97	4 90	4 83	4 76	04	*15*	7 35	7 24	7 13	7 02	6 92	05
16	4 69	4 62	4 56	4 50	4 44	03	*16*	6 82	6 72	6 63	6 54	6 45	05
17	4 38	4 32	4 26	4 20	4 15	03	*17*	6 36	6 28	6 0	6 12	6 04	04
18	4 10	4 05	4 00	3 95	3 90	02+	*18*	5 96	5 89	5 82	5 75	5 68	04
19	3 85	3 80	3 76	3 71	3 67	02	*19*	5 61	5 54	5 48	5 42	5 36	03
20	3 63	3 59	3 55	3 51	3 47	02	*20*	5 30	5 24	5 18	5 12	5 07	03
21	3 43	3 40	3 36	3 32	3 29	02−	*21*	5 02	4 96	4 91	4 86	4 81	03−
22	3 25	3 22	3 18	3 15	3 12	02−	*22*	4 76	4 71	4 66	4 61	4 56	03−
23	3 09	3 06	3 03	3 00	2 97	02−	*23*	4 52	4 47	4 42	4 38	4 34	02
24	2 94	2 91	2 88	2 86	2 83	01+	*24*	4 30	4 26	4 22	4 18	4 14	02
25	2 80	2 78−	2 75	2 73−	2 70	01+	*25*	4 10	4 06	4 02	3 98	3 94	02
26	2 68−	2 65	2 63	2 61−	2 58	01+	*26*	3 91	3 81	3 83	3 79	3 76	02
27	2 56	2 54	2 52−	2 50−	2 47+	01	*27*	3 73	3 69	3 66	3 63	3 60	02−
28	2 45	2 43	2 41	2 39	2 37+	01	*28*	3 57	3 54	3 51	3 48	3 45	01+
29	2 36−	2 34	2 32	2 30	2 28	01	*29*	3 43	3 40	3 38	3 35	3 33	01
30	2 27−	2 25−	2 23	2 21	2 20−	01	*30*	3 30	3 28	3 25	3 22	3 20	01
31	2 18	2 17−	2 15	2 13	2 12−	01	*31*	3 18	3 15	3 13	3 11	3 09	01
32	2 10	2 09	2 07	2 06	2 04		*32*	3 07	3 04	3 02	3 00	2 98	01
33	2 03	2 02	2 00	1 99	1 97		*33*	2 96	2 94	2 92	2 90	2 88	01
34	1 96	1 95	1 94	1 92	1 91		*34*	2 86	2 84	2 82	2 80	2 78	01
35	1 90	1 88	1 87	1 86	1 85		*35*	2 77	2 75	2 73	2 71	2 70	
36	1 84	1 82	1 81	1 80	1 79		*36*	2 68	2 66	2 64	2 63	2 61	

*The values are calculated for a ten fold dilution (*see text*) If 2 cc aliquots are used subtract 3 per cent from the value for 4 cc aliquots and multiply by 2

may be made immediately after addition of stannous chloride to a large series A delay of 2 hours or even longer is permissible

Calculation After calculation is made in the usual manner from the readings of the standard and unknown corrections must be applied for deviation from Beer s law and for the effects of tri

chloracetic acid and glycerophosphate * Bodansky has compiled tables which give the corrected values corresponding to given readings † The values of the check solutions, which contain neither trichloracetic acid nor glycerophosphate may be obtained from Table 1 the value of 'serum inorganic P, in mg per 100 cc is found in Table 2, under the division corresponding to the volume of aliquot used values of total inorganic P are obtained in similar manner but since the dilution in the standard procedure is 20 the table value is multiplied by 2 When dilutions of 30 or 40 are employed, the table value is multiplied by 3 or 4 respectively

A unit of phosphatase activity is defined as that amount of activity which will liberate 1 mg of P (as the phosphate ion) during the first hour of incubation under the conditions described The difference between total inorganic P after incubation and serum inorganic P, both expressed on the basis of 100 cc of serum equals units of phosphatase activity per 100 cc

VITAMIN E

At the present time there are no methods available for satisfactory determination of the status of an individual as regards vitamin E adequacy by laboratory methods

VITAMIN K

The relatively recent concept that vitamin K is essential to the proper formation of prothrombin by the liver and the maintenance of a normal blood plasma content of prothrombin has prompted the development of several methods for estimating the prothrombin content of blood plasma This in turn reflects the adequacy of vitamin K in an individual who is free of liver damage and a few rarer complications Quick[26 27] has devised a method for estimating

* When serum phosphatase activity is high the relatively large amounts of liberated inorganic phosphate retard the hydrolysis Bodansky's tables include corrections for this effect which are applied to the value of inorganic P liberated per 100 cc. of incubation A simple rule is to divide this value by 30 and add the square of the quotient to the uncorrected value Thus the correction for an observed value of 60 mg would be 4 mg making a corrected value of 64 mg If one half or one quarter hour incubation periods are employed multiply the uncorrected values by 1 82 and 3 30 respectively On rare occasions when two- or three hour incubation periods are desirable the factors 0 55 and 0 39 respectively are used

† Tables 1 and 2 are reproduced through the courtesy of Dr A. Bodansky and the P Blakiston and Son Philadelphia Pennsylvania

the prothrombin content of blood plasma that is both technically simple and rapid and is clinically satisfactory The principle utilized in this test is that if one adds an excess of calcium and an excess of thromboplastin to the plasma of oxalated blood prothrombin be comes the only variable in the reaction and the amount of thrombin formed depends on the concentration of the prothrombin present The clotting time therefore becomes a direct measure of pro thrombin content

The Determination of the Prothrombin Time of Blood Plasma

Reagents and Solutions 1 Sodium oxalate solution 1 34 Gm an hydrous sodium oxalate c p is dissolved in 100 cc of distilled water

2 Calcium chloride solution 1 11 Gm anhydrous calcium chlo ride c p is dissolved in 400 cc of distilled water (a 0 025 molar solution of calcium chloride)

3 Thromboplastin solution* Carefully strip off the pia and blood vessels from a rabbit s brain and macerate the brain in a mor tar under acetone The macerated brain tissue is now repeatedly ex tracted with acetone until a fine granular powder is obtained when dried on a small suction filter The extraction should not consume more than 10 minutes and the acetone used must not be acid This powder is now placed in ampoules 0 3 Gm to each ampoule and the ampoules are then evacuated by means of an oil suction pump for 3 minutes and finally sealed in a gas flame Prepared in this manner the thromboplastin maintains its potency for several months To prepare the solution open an ampoule add 5 cc of physiologic saline and incubate the mixture at 50°C for 15 min utes Then allow the mixture to stand until the coarse particles have settled out and a milky supernatant is obtained After opening the ampoule and preparing the thromboplastin solution deterioration occurs more readily If kept carefully stoppered and in a refriger ator it will maintain a satisfactory potency for about one week

Procedure Add 4 5 cc of venous blood to 0 5 cc of the sodium oxalate solution mix and centrifuge to obtain a clear supernatant plasma 0 1 cc of this plasma is added to 0 1 cc of the thrombo plastin solution in a small watch glass or the depression of a hang ing drop slide 0 1 cc of the calcium chloride solution is added Coincident with the addition of the calcium chloride a stop watch is started The mixture is needled about once each second and the time of formation of the first fibrin strand is recorded This is

* Thromboplastin solution is now available commercially

During the first minutes of distillation only a low flame is used As distillation advances and the boiling point of the mixture rises the heat may be increased *Distillation is continued until* from one half to two thirds of the solution has boiled away or until the first signs of bumping Most of the ammonia comes over within the first few minutes In the latter part of the distillation the delivery tube may be lifted out of the acid receiving solution as a precaution against sucking acid back into the condenser

When the distillation is complete the delivery glass tube is disconnected from the still *before the flame is extinguished* The free acid in the receiving flask is now ready for titration with the standard 0 05 N sodium hydroxide solution This is done, and the amount of 0 05 N sodium hydroxide necessary to titrate the excess acid to the end point of the indicator is recorded

In order to correct for any nitrogen present in the reagents, solutions, et cetera a blank determination must be made This is done in exactly the same manner as is outlined above except that *no* blood plasma is included All other reagents and solutions are used in the same amounts, and the procedure is carried out exactly as in the determination of the total nitrogen

Determination of plasma (or serum) albumin plus non protein nitrogen

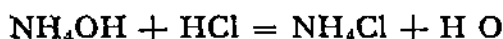
Procedure One cc of plasma is placed in a small Erlenmeyer flask, and 30 cc of the 22% sodium sulfate solution and a small crystal of thymol are added Mix, stopper the flask and allow it to stand in an incubator at 38° for three hours or longer Then filter in the incubator through a hardened filter paper * The filtrate contains the albumin and non protein nitrogen Certain plasmas do not filter clear at once In these cases it is necessary to pass the cloudy filtrate through the filter two or more times until a clear filtrate is obtained Occasionally from highly lipemic plasmas a clear filtrate cannot be obtained In such a case one may analyze the still somewhat cloudy filtrate The suspended matter appears to be fat which does not significantly affect the nitrogen figures Ten cc portions of this filtrate are analyzed by the macro Kjeldahl procedure The technics of digestion distillation, and titration are the same as described above for the determination of the total plasma nitrogen

* Funnels flasks and pipettes must be kept constantly at 37 38 Otherwise the sodium sulfate will crystallize from the solution The technique of Robinson Price and Hogden[28] eliminates this troublesome detail

Again one must make a blank determination as in the case of the total plasma nitrogen The reagents solutions and procedure are duplicated but no plasma included in the blank determination

Calculations Following the digestion distillation and titration procedures on the total plasma and albumin samples one is able to calculate the grams of nitrogen present in the respective samples and then this may be readily converted to grams of protein

By the above procedure the nitrogen present in the unknown sample of plasma has been converted into ammonia and this is distilled into a known amount of 0 05 N HCl This ammonia neutralizes its equivalent amount of acid according to the following formula



$$NH_4OH + HCl = NH_4Cl + H\ O$$

Consequently when one titrates the excess 0 05 N acid remaining after distillation is complete with 0 05 N NaOH the difference between the original total acid in cc present minus the cc of equivalent alkali necessary to titrate the excess acid equals the amount of nitrogen present in terms of cc of 0 05 N NH_4OH The grams of nitrogen present may be calculated accordingly since

1 cc 0 05 N HCl ≈ 1 cc 0 05 N NaOH ≈ 1 cc 0 05N NH_4OH ≈ 0 0007 Gm N

Obviously one must deduct from the grams of N found in the sample any nitrogen present in the reagents et cetera as found by the blank determination All this may be done by the following formula

Gm of N in sample = (A B C) × 0 0007 in which
A = Total volume in cc of 0 05 N HCl placed in distilling receiver
B = Volume of 0 05 N NaOH in cc required to titrate the excess of acid not neutralized by ammonia
C = Value of A B found in blank analysis of reagents

In order to convert the above value for grams of N in the sample into grams of N per 100 cc of plasma (grams per cent) one must take into account the dilution of the original sample In the determination of the total nitrogen this may be done by multiplying the value obtained above by 250 For albumin nitrogen the value is multiplied by 310

The value for the protein fractions represented by the total nitrogen and albumin nitrogen values obtained above may be calculated

by multiplying each by the factor 6 25 But since non protein nitrogen (N P N) is also measured by this method in both the albumin and total nitrogen determinations, this factor must be considered in the calculations The different protein fractions may therefore, be calculated as follows

Total protein* = (Total N in Gm % — N P N ** in Gm %) × 6 25
Albumin protein = (Albumin N in Gm % — N P N ** in Gm %) × 6 25
Globulin protein = Total protein — albumin protein

IRON

The laboratory diagnosis of iron deficiency consists of the combined utilization of a red blood cell count hemoglobin determination, and the determination of the volume of packed cells as measured in a sedimentation tube With this data, one can determine the presence or absence of anemia and can satisfactorily classify the type of an anemia if present, by the calculation of the mean corpuscular volume (M C V) the mean corpuscular hemoglobin (M C H) and the mean corpuscular hemoglobin concentration (M C H C)

It seems unnecessary to describe the technics for red blood cell *counts, hemoglobin determinations, and obtaining the* packed cell volume and the hematocrit values since these are standard procedures commonly employed

IODINE

No laboratory methods have been widely employed in the diagnosis of an iodine deficiency Such laboratory methods as have been proposed are not practical for general use and their interpretation is not entirely clear at the present time

* Protein determinations may be made on either blood plasma or serum as has been indicated previously The difference in the results obtained are slight the plasma values being slightly higher

** If determinations are made on plasma or serum from patients with no marked alteration in the serum N P N it is feasible arbitrarily to use the value of 0 035 (35 mg %) for N P N in the formula

*Recommended Dietary Allowances**

Food and Nutrition Board National Research Council Circular No 115

	C lories	Prote grams	Calcium gr ms	Iron mg	V tamin A I U	Thi min (B) mg	Ribo-fl vin mg	N acin (N oti nic a d) mg	Asc b c acid mg	V tamin D I U
M (70 Kg.)										
Sed tary	2500					1 5	2 2	15		
Moderately a t ve	3000	70	0 8	12	5000	1 8	2 7	18	75	†††
V ry acti	4500					2 3	3 3	23		
Woman (56 Kg)										
Sedentary	2100					1	1 8	12		
Mod rately acti e	2500	60	0 8	12	5000	1 5	2 2	15	70	†††
Very acti e	3000					1 8	2 7	18		
Pregn cy (latter half)	2500	85	1 5	15	6000	1 8	2 5	18	100	400 to 800
Lactation	3000	100	2 0	15	8000	2 3	3 0	23	150	400 to 800
Children p to 12 years										
Under 1 y †	100/Kg.	3 to 4/Kg	1 0	6	1500	0 4	0 6	4	30	400 to 800
1–3 y rs††	1200	40	1 0	7	2000	0 6	0 9	6	35	†††
4–6 years	1600	50	1 0	8	500	0 8	1 2	8	50	
7–9 y ars	2000	60	1 0	10	3500	1 0	1 5	10	60	
10–12 years	2500	70	*1 2*	*12*	*4500*	*1 2*	*1 8*	1	75	
Children ov 12 years										
G ls 13–15 years	2800	80	1 3	15	5000	1 4	2 0	14	80	†††
16–20 years	2400	75	1 0	15	5000	1 2	1 8	12	80	
Boys, 13–15 y rs	3200	85	1 4	15	5000	1 6	2 4	16	90	†††
16–20 years	3800	100	1 4	15	6000	2 0	3 0	20	100	

*Tentative goal toward which to aim in planning practical dietaries can be met by a good diet of natural foods Such a diet will also provide other minerals and vitamins the requirements for which are less well known

**1 mg thiamin equals 333 I U 1 mg ascorbic acid equals 20 I U

***Requirements may be less if provided as vitamin A greater if provided chiefly as the pro vitamin carotene

†Needs of infants increase from month to month The amounts given are for approximately 6 8 months The amounts of protein and calcium needed are less if derived from breast milk

††Allowances are based on needs for the middle year in each group (as 2 5 8 etc.) and for moderate activity

†††Vitamin D is undoubtedly necessary for older children and adults When not available from sunshine it should be provided probably up to the minimum amounts recommended for infants

Further Recommendations Adopted 1942

The requirement for *iodine* is small probably about 0 002 to 0 004 milligram a day for each kilogram of body weight This amounts to about 0 15 to 0 30 milligram daily for the adult This need is easily met by the regular use of iodized salt its use is especially important in adolescence and pregnancy

The requirement for *copper* for adults is in the neighborhood of 1 0 to 2 0 milligrams a day Infants and children require approximately 0 05 per kilogram of body weight The requirement for copper is approximately one tenth of that for iron

The requirement for *vitamin K* is usually satisfied by any good diet Special consideration needs to be given to newborn infants Physicians commonly give vitamin K either to the mother during pregnancy or to the infant immediately after birth

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